Vol. 35, No. 2 June 1948

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

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CAMBRIDGE UNIVERSITY PRESS
CAMBRIDGE, AND BENTLEY HOUSE, LONDON
AGENTS FOR U.S.A.
THE UNIVERSITY OF CHICAGO PRESS
CANADA AND INDIA: MACMILLAN

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THE TOXIC ACTION OF MOLYBDENUM IN RELATION TO SOILS AND CROPS

BY WINIFRED E. BRENCHLEY, D.Sc.
Rothamsted Experimental Station

(With Plate 2)

The harmful effect of molybdenum on animals is well established, but there is comparatively little knowledge of the conditions in which molybdenum is poisonous to plants. Obvious differences in response to molybdenum poisoning in different soils have been previously noted, but no adequate explanation has been put forward.

Tomatoes grown in ordinary loam showed little outward sign of poisoning with heavy doses of sodium molybdate, but some depression of crop occasionally occurred, especially when the dose was divided into early and late treatments. The response varied with season and variety. On certain light and fen soils the plants were killed at an early stage with the heavier dressing of molybdate, and seriously injured with the lighter dose, the leaves showing the golden colour characteristic of molybdenum poisoning in various plants.

The reaction of different crops varied considerably in the same soil with similar treatments. On old cucumber soil tomatoes showed no sign of toxicity even with the heavy dressing, flax was progressively damaged with increasing doses, while *Solanum nodiflorum* was most seriously affected even with the lighter dressing of molybdate. It was impossible to predict the reaction between soil, poison and crop without actual experiment.

The growth of flax was greatly impeded on a manganese-deficient fen soil, and the molybdenum toxicity was masked in consequence. When the deficiency was corrected the poisonous effect of molybdenum on this soil was very marked, even with the lower dressing.

The composting of loam with peat usually, but not invariably, resulted in a reduction of the toxicity of molybdenum. The different results obtained with several crops on a variety of soils still yield no definite clue to the factors which determine the relative toxicity of molybdenum to plant growth and such points as soil acidity, response to climatic conditions and nutritive deficiencies are among those which need further investigation.

HISTORICAL

During recent years increasing attention has been paid to molybdenum in its relation to the health and well-being of plants and animals. Molybdenum is a normal constituent of many plants, usually occurring in very small and varying amounts for any one species. Bertrand (1939) found a specially high content in leguminous plants and crucifers, and Ferguson, Lewis & Watson (1940) gave the average amounts of the element in the dry matter of various plants as: wild white clover, 0·0095%; wild red clover, 0·0089%; Yorkshire fog, 0·0069%; perennial ryegrass, 0·0011%.

This was confirmed by Lewis (1943*a*, *b*), who showed that basic fertilizers increase the molybdenum uptake of grasses on acid soils. Calcareous and alkaline soils containing about 20 p.p.m. or more molybdenum in the surface horizon are

potentially teart, i.e. are liable to cause 'teart' disease in animals if the pastures are grazed. The molybdenum content of newly sown grasses is low, but increases with age. The important fact is that plants are able to absorb without harm quantities of molybdenum that are definitely poisonous to animals. The amount of molybdenum in the soil increases with depth, and the uptake of the element may be greater, the greater the depth of root penetration.

The molybdenum content of a number of soils, chiefly French, was found to vary from 4·3 to 69 mg./kg. soil (Bertrand, 1940*a*). No apparent correlation appears to exist between the total or HCl-soluble molybdenum of the soil and that of the herbage. Stephens & Oertel (1943) stated that in pot cultures with shaley clay-loam soils in Australia there was an increase in the yield and the molybdenum content of perennial ryegrass and white clover for each pH value tested in molybdenum-treated pots.

The apparent affinity of leguminous plants for molybdenum has been confirmed by many workers. Vinogradova (1943) found an average of more than ten times as much molybdenum in leguminous seeds as in those of other plants, and two to five times as much in the nodules of clover, vetch and lupin as in their seeds. This was held to support the hypothesis that molybdenum in root nodules plays an important part in nitrogen fixation by *Azobacter*. Dmitriev (1938*a*, *b*, 1941) obtained a beneficial effect of molybdenum on the seed yield of red clover, provided it was applied with boron in the presence of lime, phosphate and potassium. Alone, the element was ineffective or even harmful on podzol soils, but on limed podzols the action of boron was enhanced by it, the optimum amount of molybdenum in pot experiments being 1–5 mg./kg. soil. Larger quantities decreased the seed yield. Working with water cultures Bertrand (1940*b*) found that leguminous plants can grow normally in media containing a wide range of molybdenum, but the dry weight of peas varied with the amount of molybdenum supplied, maximum weight being obtained with 0·04 mg./l. The molybdenum content of the dry matter of the same species from different places may vary greatly, as from 0·82 to 2·28 mg. Mo/kg. in white lupin, 0·674–1·23 in wild carrot, and 1·78–2·70 in plantain. When molybdenum dressings are applied to pastures the molybdenum content of the herbage is increased, but this is entirely due to the effect of clover (Teakle, 1944). The presence of adequate phosphate and other nutrients are essential if beneficial results are to be obtained from this treatment (Anderson, 1946).

Of recent years claims have been made that traces of molybdenum are essential for the proper development of certain plants, though it may be that this is true only in particular conditions of environment and nutrition which have not yet been fully worked out. Arnon & Stout (1939) working in California overcame deficiency symptoms in tomatoes with 1 part in 100,000,000 molybdenum applied as molybdic acid, no other element having the same effect. Injury from excess only occurred if the concentration exceeded 10 p.p.m. Mo. After several years' intensive work with lettuce Warington (1946) was forced to the conclusion that 'in spite of the occasions

on which no response was obtained molybdenum plays an important part in maintaining the normal health of lettuce. Such striking proof was obtained so frequently in support of this view that the negative results can only be regarded as exceptional.'

So much attention has been focused on the harmful effect of molybdenum on animals, and its possible beneficial effect on plants, that the other side of the question has received less attention, and our knowledge of the conditions in which this element is poisonous to plants is very slender. Warington (1937) found that *Vicia faba* and barley, grown on ordinary loam, were insensitive to quite heavy dressings of sodium molybdate, no reduction in growth being obtained with 4 g./10 kg. soil, though at this strength and beyond barley tended to weaken from the base and fall over, a condition confirmed recently in flax, and described later (p. 149). *Solanum nodiflorum* succumbed to a lower dressing on an old cucumber soil, but was less badly affected when the same soil was mixed with the Rothamsted loam. Tomatoes were nearly all killed by 4 g. sodium molybdate per 10 kg. cucumber soil, but made fair growth with half the dose. In contrast to this, Elze (1940) in Palestine found that in a poor sandy soil toxic symptoms appeared in tomatoes with dressings that permitted good development in the Rothamsted experiments. He suggested that this was probably due to differences in the amounts and absorptive capacities of the soils used in the two sets of experiments.

Experimental

In view of the obvious differences in the responses to molybdenum poisoning on various soils, a series of experiments was started to investigate the factors responsible for the discrepancies. For five years, from 1942 to 1946, tomatoes were grown as a single experiment with certain variations from year to year. During 1945 and 1946 the work was extended to a range of very different soils, tomatoes again being used, as they are supposed to have a special need of molybdenum, *Solanum nodiflorum* being added because it had shown morphological peculiarities in the presence of the element (Sheffield, 1934), and flax because its nutritional needs were being investigated in other connexions.

Tomatoes

In the main series (1942–6) red earthenware pots of standard size, holding about 10 kg. loam, were used throughout, with the same mixture of fertilizers, the same cultural treatment and a scheme of molybdenum dressings that varied from year to year, but devised within a framework which enabled comparisons to be made throughout the period of experiment. Heavy dressings of fertilizers were used, providing sufficient slow-acting nitrogen in the form of powdered hoof to carry the plants to maturity without any top dressings. The quantities were, per pot: 60 g. hoof meal; 15 g. superphosphate of lime; 30 g. potassium sulphate; 20 g. calcium carbonate, precipitated. In 1945 and 1946 magnesium sulphate was added during July at the rate of 5 g. per pot, as signs of magnesium deficiency became evident.

The basal soils used varied, being Harpenden allotment soil in 1942 and 1943,

a mixture of Harpenden and Redbourn loam in 1944, and top-spit loam from Highgate, in the London area, in 1945 and 1946. Composts were made every year, with dead leaves in 1942 and 1943, and with peat later. In the last two years the straight soils mixed with a proportion of sand were also tested. The compost was made up, by volume, of 7 parts loam, 2 parts sand, 3 parts decayed leaves or peat. The effect of treatment was estimated by the weights of fruit produced. Tomatoes were harvested about twice a week, counted and weighed, being gathered when they were sufficiently coloured to ripen off fully within a few days from picking. Observations showed no apparent differences in the growth of the plants in relation to the molybdenum dressings, and it was not possible to pick out those that were heavily treated from the randomized plants. The number of replicates varied from three to eight in the 5-year series. Usually two varieties were grown, Potentate and Kondine Red, in two greenhouses giving somewhat different environmental conditions, which apparently had no effect on the results (Tables 1 and 2).

The cropping was satisfactory, ranging from an average of $5\frac{1}{2}$ to 10 lb. per plant, varying with season as well as with treatment. The standard errors were relatively high, as was to be expected with such a variable crop and the small number of replicates. Even so, positive response to molybdenum poisoning was obtained in certain cases, chiefly with Potentate plants in compost. With Potentate in 1943 there was a considerable reduction of crop with 4 g. sodium molybdate given in two doses, the response being less, but still significant, when all the poison was applied at the time of potting. Similarly in 1944 the toxic effect was greatest with 2 g. of molybdate

TABLE 1. *Potentate. Fresh weight of fruit; means for each treatment in g.*

Treatment sodium molybdate (g.)	1942	1943	1944	1945		1946	
				Soil	Compost	Soil	Compost
Control, no Mo	4250	4488	3458	3324	3883	3995	4338
0·25 early	3980	—	—	—	—	—	—
0·5 early	4186	—	—	—	—	—	—
0·5 late	4134	—	—	—	—	—	—
0·5 early + 0·5 late	—	—	3656	—	—	—	—
1·0 early	4301	—	3645	—	—	—	—
1·0 late	3978	—	3662	—	—	—	—
1·0 early + 1·0 late	—	—	2855	—	—	—	—
2·0 early	—	4413	3519	3211	3877	—	—
2·0 early 1·0 late	—	—	—	—	—	3770	4405
2·0 late	—	—	2687	3280	3934	—	—
2·0 early + 2·0 late	—	3203	—	3126	3887	—	—
4·0 early	—	3707	—	—	—	3907	—
4·0 early 2·0 late	—	—	—	—	—	—	3789
S.E.	331	206	221	182		188	

applied late, the poisoning being less severe, but still significant when 1 g. was given both early and late. The only other significant decrease was in compost in 1946 with 4 g. molybdate applied early, followed by another 2 g. later. No evidence of toxicity appeared in the straight soils, nor in the composts in 1942 and 1945. With

Kondine Red the only significant result was in 1943 with 1 g. molybdate early and 2 g. late. It would thus appear that in soils and seasons when molybdenum poisoning was evident, the effect was heightened when part, at least, of the dressing was given when the plants had made considerable growth and were within a week or two of producing ripe fruit.

TABLE 2. *Tomato. Kondine Red. Fresh weight of fruit, means for each treatment in g.*

Treatment sodium molybdate (g.)	1942	1943	1944	1945	
				Soil	Compost
Control, no Mo	4254	3911	3452	2830	3801
0·5 early	4176	—	—	—	—
0·5 late	3645	—	—	—	—
0·5 early + 0·5 late	—	—	3172	—	—
1·0 early	3936	3737	3849	—	—
1·0 late	4224	—	3217	—	—
1·0 early + 1·0 late	—	—	3373	—	—
1·0 early + 2·0 late	—	3396	—	—	—
2·0 early	4197	3981	3359	2539	3592
2·0 late	—	—	3296	2481	3671
2·0 early + 2·0 late	—	—	—	2634	3363
S.E.	177	147	222	202	

When the scope of the work was extended to include a larger range of soils the only suitable pots available were small glazed pots holding from $4\frac{1}{4}$ to $6\frac{1}{2}$ lb. according to the soil. These were excellent for flax although too small for the satisfactory growth of tomatoes and *Solanum*, but they had to be used for the preliminary trials, made in duplicate, to gain an insight into the way the work should be developed. Table 3 gives the soils used for all crops in 1945. The old cucumber

TABLE 3. *Soils used for pot cultures. Season* 1945

Soil	Wt. of soil (lb. per pot)	Chalk added (g. per pot)	N in p.p.m. of samples as received
(*a*) Allotment	$5\frac{3}{4}$	None	63·2
(*b*) Allotment compost	$5\frac{1}{2}$	None	82·1
(*c*) Woburn Farm	$6\frac{1}{2}$	1·5	18·8
(*d*) Woburn compost	5	1·0	57·4
(*e*) Rothamsted Farm	$6\frac{1}{2}$	None	14·3
(*f*) Harpenden Common	$6\frac{1}{2}$	15·0	20·2
(*g*) Isleham Fen	$4\frac{1}{4}$	None	Not received in time
(*h*) Old cucumber	$5\frac{1}{2}$	None	327·6

soil had been stored in a bin for several years, and the allotment soil was from old and very well-cultivated allotments in Harpenden. With soils (*a*), (*e*), (*f*), (*h*), 90% soil and 10% sand were used, while (*b*) and (*d*) were composted with peat. The Fen and Woburn soils were used without further treatment.

The initial manuring was 0·55 g./pot of monohydrogen potassium phosphate, and 0·943 g. sulphate of ammonia, applied in solution and well mixed with the soil. This was insufficient for tomatoes, which received extra top dressings of the full amount

on three occasions and of half the amount on six other dates. In addition, 1 g. magnesium sulphate per pot was given early in July.

At the time of planting the nitrogen available per pot for the different soils was calculated from the determinations of the soil nitrogen with the addition of that supplied by the fertilizers.

TABLE 4

	N in soil (g. per pot)	N from fertilizer (g. per pot)	Total N (g. per pot)
(*a*)	0·164	0·20	0·364
(*b*)	0·205	0·20	0·405
(*c*)	0·055	0·20	0·255
(*d*)	0·130	0·20	0·330
(*e*)	0·042	0·20	0·242
(*f*)	0·060	0·20	0·260
(*g*)	Less than 0·124*	0·20	Less than 0·324
(*h*)	0·817	0·20	1·017

* This determination was accidentally lost. The figure given was a rough estimate of about half the nitrogen present after 2 months' incubation at 30° C.

Owing to difficulties of supply in 1945 sodium molybdate was unavailable when required, so for that one year potassium molybdate was substituted with the addition of enough potassium chloride to the controls and low molybdenum set to equalize the amount of potassium given. It was difficult to determine whether the rate of molybdenum application should be a direct relation to the amount of soil in the pots, when compared with the larger quantities in the red earthenware pots. A compromise was reached and 1·5 g. molybdate was given for the heavy dressings and 0·75 g. for the lower rate. Some weeks after the first application an additional half-dressing of molybdate was applied to all the treated pots.

Tomato seedlings, var. Potentate, were planted out early in April and allowed to become fully established before the molybdenum was given early in May. Growth varied somewhat between the soils, but no harmful effect of molybdenum was shown except in the Fen soil, although various plants showed the characteristic yellow colour, which, however, did not develop further. Ten days after application of the heavy dressing the lower part of the leaflets in the Fen soil were almost gold

TABLE 5. *Tomatoes. Potentate.* 1945. *Weight of fresh fruit. Means of two plants*

Soil	Control (g.)	Low Mo (g.)	High Mo (g.)
Allotment soil	860	831	894
Allotment compost	782	805	860
Woburn soil	831	1009	536
Woburn compost	1053	917	911
Rothamsted Farm	788	998	961
Harpenden Common	807	719	894
Isleham Fen	676	943	—
Cucumber	1000	984	956

S.E. mean 89.

in colour. Growth was very poor and the plants remained tiny and became very unhealthy, the leaves being very small, flaccid, and showing the bright yellow colour characteristic of molybdenum poisoning. At this time the plants with the lower dressing were also affected to some degree, the leaves having narrow blades and becoming yellowish at the base. By the beginning of June the heavily poisoned plants were dead, but the others had made a good recovery though the lower leaves were bright gold in colour. Except for the killing of the plants in Isleham Fen soil with high molybdenum, much weight cannot be placed on these results, owing to the low replication and the natural variability of the plants. The high dosage was, however, apparently harmful in the straight Woburn soil, though not in the compost, and this observation was borne out in the next year's work (Table 5).

In 1946 this experiment was repeated in larger pots holding 10 kg. ordinary loam, bringing the conditions nearer to those in the long-term tests already described. Woburn soil and Isleham Fen soil were again available, two other fen soils were introduced, and a top-spit loam used instead of the allotment soil. Littleport Fen

TABLE 6. *Soil used for pot cultures.* 1946

Soil	pH
(*a*) Top-spit loam	5·76
(*b*) Top-spit loam composted	5·00
(*c*) Woburn	6·90
(*d*) Woburn compost	5·60
(*e*) Isleham Fen	7·55
(*f*) Littleport Fen	6·20
(*g*) Waterbeach Fen	4·78

soil was chosen as it had given very heavy crops of sugar beet, potatoes and wheat during the three previous years without any signs of the manganese deficiency known to occur in Isleham Fen. Waterbeach Fen is distinctly acid, but no addition of chalk was made to it in this experiment, in order to get a comparison with the other fen soils in their natural state. 10 g. of calcium carbonate was added per pot to the top-spit loam and its compost (Table 6). In this case inorganic nitrogenous fertilizers were again used instead of the slow-acting hoof meal. It was hoped to introduce fertilizers totalling those where hoof meal had been used, but an equivalent amount of ammonium sulphate supplied all at once seemed inadvisable. Therefore 6·6 g. monohydrogen potassium phosphate was given, supplying all the phosphate required and part of the potassium, with 12 g. ammonium sulphate as a start. Then at intervals 4 g. ammonium sulphate and/or 3 g. potassium sulphate was given to build up the fertilizer requirements. The total ultimately supplied was 28 g. ammonium sulphate (equivalent to 42 g. hoof meal), and the equivalent of 15·6 g. potassium sulphate, in addition to the phosphate supplied at the beginning. This manuring proved adequate, and allowed the different reactions of the soils to show up clearly.

The influence of molybdenum on the growth of tomatoes varied greatly with the soil. With the top-spit loam and compost, and with Waterbeach and Littleport Fen soils the largest quantity of molybdenum used was inocuous, the treated plants being

as good as the controls within the limits of experimental error. On the Woburn soil the plants were very quickly poisoned, as within 10 days those receiving the heavy dose were unthrifty and 4 days later were practically dead. Plants given the lighter dressing over the same period showed great variability, some being dead, some very poor and some fairly healthy. The living plants were characteristically constricted at the base of the stems, with numbers of adventitious root initials above the constrictions. Three weeks later one plant had made a considerable recovery, but the others were all dead, the foliage being flaccid and drooping, dying from the apices of the leaflets. By this time the root initials had appeared half-way up the stems, and these poisoned plants had developed a few tiny fruits which were eventually harvested.

The composting of the Woburn soil with peat mitigated the toxic action very considerably. With the lower dressing only one plant showed any ill effects within the first 3 weeks, but no root initials were developed and after a time the plant made a good recovery and produced a crop of fruit that was not much below its replicates, the latter being as good as the controls in the same compost. The action of the high molybdenum was extremely variable and resembled that of the lower dose on the straight soil, three plants being killed and one being as well developed as the controls. An interesting point with this plant was the large number of tiny fruits that were produced, twenty-six weighing less than 10 g. each and totalling only 123 g., the maximum on any other plant in this experiment being eight totalling 54 g.

On the Isleham Fen soil, which is short of available manganese, signs of deficiency began to appear in the untreated controls 6 or 7 weeks after planting, as well as in those receiving molybdenum. Light green areas or brown lesions were the early signs, and the leaves soon became characteristically speckled yellow and brown. As growth proceeded the younger top leaves were at first green, while the lower leaves became particoloured or died off. The toxicity of the molybdenum increased progressively with the dose, but in no case was it as fatal as in the Woburn soil. Here again the sickly plants developed the adventitious root initials. The worst plant with the heavy dressing fell over at the base, the stem curled backwards and

TABLE 7. *Tomatoes. Potentate.* 1946. *Weight of fresh fruit. Mean of four plants*

Soil	Control (g.)	Low Mo (g.)	High Mo (g.)
Top-spit loam	2969	2938	3222
Top-spit compost	2917	2944	3017
Woburn soil	2581	511	—
Woburn compost	2230	2263	779
Isleham Fen	2547	1213	782
Littleport Fen	3405	3151	3162
Waterbeach Fen	1947	1990	2007

the leaves varied in colour from greenish to bright yellow, indicating severe molybdenum poisoning. The total weight of fruit produced in 1946 was much heavier than in 1945, on account of the larger amount of soil and the better fertilizing (Table 7). On the loam soil and compost, and on the Littleport and Waterbeach Fen soils molybdenum had no effect with either dose. On the manganese-deficient Isleham Fen soil there was a steady decrease in weight with increasing molybdenum, but the heavier dose did not quite kill the plants, as in the previous year. On the Woburn soil, on the contrary, the toxic action was much stronger in 1946 with a heavy decrease in crop with the lower dressing and a complete kill with the heavier dose. In the compost, too, the heavy dose was very poisonous, whereas previously it was inocuous. The reverse response to toxicity on Woburn and Isleham Fen soils in the two years weakens the idea that the difference was due to variation in the relative rates of application of the poison, as if this had been so the change in response would have been the same in both years, and once again one is brought up against the 'unknown factor' which influences the action of molybdenum on growth.

Solanum nodiflorum

The seed required considerable heat to encourage germination, but after the seedlings were pricked out growth continued well in the moderate temperatures available in the pot-culture house. Four or five weeks after sowing the seedlings were large enough to be transplanted to their respective soils, the first molybdenum treatment being given about a week later. The trucks carrying the pots were shaded with light muslin until the plants were well established.

1945 *experiment*

The test was made in duplicate in the small glazed pots, and within 11 days all the plants in *cucumber* soil treated with both levels of molybdenum were seriously affected, and were very small with olive or multicoloured leaves. These made no recovery and were soon dead (Pl. 2, fig. 1). Few signs of toxicity were apparent with the other soils at this date, except for a certain number of multicoloured leaves, and also a reduction in size in the treated Fen soil, and, rather late, in the heavily dosed Woburn soil. After the second treatment with molybdenum, poisoning became more evident. On the allotment soil (Pl. 2, fig. 3) straight and composted, and on the Woburn soil and compost the lower leaves turned golden yellow, a very characteristic sign of molybdenum toxicity. Reduction in size became more accentuated on all the soils except the two composts, though the lower dressing had little apparent effect on Woburn soil. A noticeable feature was the difference in individual response with the higher dose, as on some soils one plant was killed or very seriously damaged, whereas the other was much less affected, if at all (Pl. 2, fig. 2). This is best seen by comparing the final weights, which are shown separately for these cases in Table 8.

There was a general tendency for plants in most soils to be a darker colour in the

presence of molybdenum, as is frequently seen with lettuces in other experiments. The heavier dressing was more definitely toxic than with tomatoes.

TABLE 8. 1945. Solanum nodiflorum. *Dry weights* (*g.*). *Mean of two plants*

O = Control; L = low Mo; H = high Mo.

	Shoot			Fruit			Total		
Soil	O	L	H	O	L	H	O	L	H
Allotment	19·05	16·77	11·75	12·82	9·68	7·55	31·87	26·45	19·30
Allotment compost	18·30	21·02	18·45 0·14	12·65	10·84	11·47 —	30·95	31·86	29·92 0·14
Woburn	13·55	13·27	1·42	11·20	9·43	0·16	24·75	22·70	1·58
Woburn compost	12·08	15·90	14·25 2·99	12·00	11·86	11·73 0·08	24·08	27·76	25·98 3·07
Rothamsted Farm	12·13	9·80	8·45	9·05	8·21	5·42	21·18	18·01	13·87
Harpenden Common	12·62	11·00	10·80	21·20	9·94	7·43	24·82	20·94	18·23
Isleham Fen	15·30	9·70 0·32	5·45 0·40	11·12 —	5·52 —	1·89 —	26·42	15·22 0·32	7·34 0·40
Cucumber	39·50	0·27	0·39	27·78	—	—	67·28	0·27	0·39

The plants were harvested when almost all the fruits were fully ripe and deep black in colour. There was a general progressive reduction in dry weights with treatment to a greater or less degree in all the soils except the two composts, though, curiously, one plant in each of the heavily treated composts, as well as in the Fen soil, died or made little development. As the test was made only in duplicate, it is not possible to attach any significance to the rather higher shoot growth in the two composts with the low dose of molybdenum. In the Fen soil all the leaves in the controls as well as in the treated plants were covered with pale green spots, probably a result of manganese deficiency. One noticeable feature of the growth of the control plants was the high dry weight of the plants in cucumber soils, more than twice as great as that in any other soil, doubtless associated with the high nitrogen content (Table 3). The nitrogen content must have been a definite limiting factor to growth in 1945, but this apparently did not affect the molybdenum reaction, which followed the same lines in the soils that were again tested the next year.

1946 *experiment*

In 1946 only five soils were tested, a good top-spit loam replacing the allotment soil, and the Woburn and Isleham Fen soils being again used, with four replicates for each treatment.

Ten days after the first addition of molybdenum four plants with the higher dressings were seriously injured, and were dead 4 days later, two in the top-spit loam, and one each in Woburn compost and Fen soil. By this time other plants

were also showing symptoms of toxicity indicated by multicoloured or variegated leaves:

Soil	Plants showing toxic effects: Low Mo	High Mo
Top-spit loam	—	1
Top-spit compost	1	2
Woburn soil	2	2
Woburn compost	1	2
Isleham Fen	1	3

Some of these plants died rapidly, some remained weak, and some recovered or the damage did not proceed further. Poisoned plants were very dark and retarded, with their leaf edges rolling up, while some plants became brittle at the base, and tended to fall over easily. In fact, it was necessary to stake certain plants in order to bring them to maturity without breakage. In contrast to the 1945 plants, the second application of molybdenum did not increase the toxic action, possibly because of the longer interval between treatments, 6 instead of 3 weeks, as in the previous year.

Throughout the series not a single plant died among the controls, though some succumbed in all the straight soils treated with molybdenum and a few also in the composts. The mean dry-weight figures relate only to the plants still living at harvest that had made appreciable growth before they died, so that in some cases the mean figures fail to show the true damaging effect of molybdenum. Great care was taken in making the molybdenum applications to avoid letting the solutions touch the plants, and in most cases, if not all, the pots were watered with distilled water immediately after treatment, to wash the molybdenum below the surface. In this series the roots were separated from the soil as well as possible by hand, thoroughly washed and dried, the final figures showing the same trend of behaviour as in the shoots and fruit, though the degree of response varied in different parts of the plants.

TABLE 9. Solanum nodiflorum. 1946. *Dry weights (g.). Mean of four plants*

O = control; L = low Mo; H = high Mo.

Soil	Shoot: O	L	H	Fruit: O	L	H
Top-spit loam	41·24	37·30	20·55	36·64	28·12	12·92
Top-spit compost	45·52	34·40	34·70	40·22	26·86	23·36
Woburn	34·16	39·40	15·99	28·20	27·11	7·00
Woburn compost	44·61	45·07	48·30	36·56	32·99	27·23
Isleham Fen	18·89	6·18	1·49	19·33	2·84	0·02
	Root: O	L	H	Total: O	L	H
Top-spit loam	12·19	11·00	4·19	90·07	76·42	37·66
Top-spit compost	13·80	9·62	8·38	99·54	70·88	66·44
Woburn	15·10	11·35	1·10	77·46	77·86	24·09
Woburn compost	17·84	13·79	12·75	99·01	91·85	88·28
Isleham Fen	5·17	1·42	0·07	43·39	10·44	1·58

The larger supply of available nutrient was reflected in the yields of the control plants, which were much heavier than in 1945 in the loam and Woburn soils and composts (cf. Tables 8 and 9). The relative increase was somewhat less in the Isleham Fen soil, possibly as a result of the strong manganese deficiency. Allowing for this, the general behaviour of the soils and their composts followed the same trend in both years.

The most convincing way of demonstrating the relative action of molybdenum in these different soils and seasons is by expressing the dry weights of the molybdenum-treated plants as percentages of the untreated controls in each case. These figures, when read in conjunction with the actual dry weights and the observations made on the morphological features of the plants during growth, give a good picture of the comparative action of molybdenum on plants under different conditions of environment (Tables 10 and 11). The toxic action of molybdenum was in general more marked on

TABLE 10. Solanum nodiflorum. 1945. *Dry weights as percentage of controls. Means of two plants*

O = control; L = low Mo; H = high Mo.

	Shoot			Fruit		Total	
Soil	O	L	H	L	H	L	H
Allotment	100	88·03	61·68	75·51	58·89	82·99	60·56
Allotment compost	100	114·86	100·82 0·77	85·69	90·67 —	102·94	96·67 0·45
Woburn	100	97·93	10·48	84·20	1·43	91·72	6·38
Woburn compost	100	131·62	117·96 24·75	98·83	97·75 0·67	115·28	107·89 12·75
Rothamsted Farm	100	80·79	69·66	90·72	59·89	85·03	65·49
Harpenden Common	100	87·16	85·58	81·48	60·90	84·39	73·45
Isleham Fen	100	61·39 2·03	35·62 2·53	49·64 —	17·00 —	57·61 1·21	27·79 1·51
Cucumber	100	0·68	0·99	—	—	0·40	0·58

TABLE 11. Solanum nodiflorum. 1946. *Dry weights as percentage of controls. Means of four plants*

O = control; L = low Mo; H = high Mo.

	Shoot			Fruit		Root		Total	
Soil	O	L	H	L	H	L	H	L	H
Top-spit loam	100	90·45	49·83	76·75	35·26	90·24	34·37	84·85	41·81
Top-spit compost	100	75·57	76·23	66·78	58·08	69·71	60·72	71·21	66·75
Woburn	100	115·34	46·81	96·13	24·82	75·17	7·28	100·52	31·10
Woburn compost	100	101·03	108·27	90·24	74·48	77·30	71·47	92·77	89·16
Isleham Fen	100	32·72	7·89	14·69	0·10	27·47	1·37	24·06	3·64

the fruit than on the shoot, the degree of difference varying considerably with the soil. In the very rich cucumber soil, really a mixture of soil and manure with a high percentage of nitrogen, the poisonous action of molybdenum was the most marked, as even with the low dressings the plants were killed within a fortnight. On the

Woburn soil the harmful effect was very great on the rather poor plants in 1945, the dry weight of the shoots only being 10·48% of the controls and the fruits only 1·43% with the heavy dose of the poison. With the better developed plants the next season 46·81 and 24·82% of the dry weight of the respective controls were produced, though there is no definite proof that the better nutrition increased the ability of the plants to resist the poison and develop more fruit.

The addition of peat to the heavy allotment soil and the sandy Woburn soil to form composts caused a considerable reduction of toxicity in all parts of the plant with the heavy dose of molybdenum, and usually with the lighter dressing also. With the top-spit loam in 1946 the peat apparently somewhat increased the toxicity of the lighter dressings, but as some of the treated plants in the straight soil died during the experiment the mean weight of the remaining plants may have been unduly heavy, as they were individually strong enough to withstand the action of the poison. The most striking effect of the peat was on the roots of the Woburn plants with high molybdenum where the relative percentage of dry matter in terms of the control was raised from 7·28 to 71·47, a tenfold increase, whereas the shoots and fruits of the same plants were only increased three and twofold respectively.

In the few cases where the percentage weight of the treated plants exceeded that of the controls it is not possible to assume that there had been any stimulation due to molybdenum, owing to the very small number of replicates, and to the fact that in several cases the higher figure relates to a single plant only, on account of the death of the duplicate. The reductions attributed to molybdenum are substantiated by the further decrease caused by a higher dose of the poison, and by the visual signs of toxicity shown during growth.

Flax

The flax experiments owe much to Major Searle and Dr Davin, of the Norfolk Flax Establishment, who provided the pedigree Liral Prince seed, and made complete fibre determinations of the 1946 crop. The seed was Nomersan dressed, sown at thirty-five seeds per pot, thinned gradually to an optimum of twenty-five. Working on previous experience no additional fertilizer was given.

1945 *experiment*

Seeds were sown on 23 April, but germination was somewhat slow and irregular, so the heavier watering was begun within 8 days, after which germination and growth levelled up rapidly. Many seeds in the cucumber soil either failed to germinate, or died early, and the final number per pot was rather lower for this soil than the others. In the early stages the best plants were in the Woburn and Fen soils, but this advantage did not persist.

Molybdenum dressings were applied 23 days after sowing, and an extra half-dressing 3 weeks later. The development of the control plants varied considerably in the different soils. In spite of the partial failure of germination those in cucumber soil

were throughout the strongest and darkest, though the advantage due to the extra nitrogen in this soil was less great than with *Solanum*. In the soils and composts from the allotment and Woburn the controls were also good and dark, but in the others they remained smaller and paler. Apart from differences in size and dry weight the development of the untreated controls was normal, except in the Isleham Fen soil, where they were discoloured, distorted and showed obvious signs of malnutrition, later proved to be due to manganese deficiency. The composting of the allotment soil did not significantly improve the dry weights of the control plants, although it was an advantage in the Woburn soil, especially in the straw (Table 12). The yield in cucumber soil was higher than any of these, but with all the others there was a heavy drop, somewhat accentuated in the Isleham Fen soil.

TABLE 12. *Flax.* 1945. *Dry weights (g.). Mean of two pots*

O = control; L = low Mo; H = high Mo.

	Straw			Seeds			Total		
Soil	O	L	H	O	L	H	O	L	H
Allotment	13·40	11·02	10·22	4·34	2·94	2·53	17·74	13·97	12·75
Allotment compost	13·70	12·87	12·25	4·97	4·41	4·01	18·67	17·29	16·26
Woburn	12·02	8·45	8·35	3·71	2·81	2·60	15·73	11·26	10·95
Woburn compost	14·45	12·30	11·52	4·38	4·35	3·46	18·83	16·65	14·99
Rothamsted Farm	9·05	8·25	8·80	2·81	2·59	2·36	11·85	10·84	11·16
Harpenden Common	9·37	9·92	9·35	3·16	2·91	2·72	12·53	12·83	12·07
Isleham Fen	8·40	4·20	3·55	1·41	0·03	0·01	9·81	4·23	3·56
Cucumber	16·65	6·32	2·30	4·70	1·00	0·06	21·35	7·32	2·36
	S.E. 0·70			S.E. 0·22					

The characteristic signs of molybdenum poisoning were a golden yellow colour of the lower leaves and a tendency to constriction of the base of the stems, causing some of the plants to lean or even fall over. The golden colour was apparent in the cucumber, Woburn, Rothamsted and Fen soils, and the falling in the Woburn compost, Harpenden Common, and Fen soils. In the allotment soil the molybdenum-treated plants were somewhat shorter and paler than the controls, but when this soil was composted with peat this effect was much less marked, as although the molybdenum-treated plants were slightly shorter the colour was as good as that of the controls. In most cases there was a steady drop in total dry weight with increasing doses of molybdenum, but this was much less marked in the allotment compost. The decrease was manifested both in straw and seeds, though to varying degrees. On the Rothamsted and Harpenden Common soils, on the other hand, the variations in dry weights were not significant. In the seeds the drop in weight with increasing doses of molybdenum was due either to an adverse effect of molybdenum on flower production or on seed development where flowers had been produced. This was of general occurrence except in Woburn soil and compost, where the straw was more adversely affected than the seeds with the lower dose of molybdenum, while the heavier dose had a similar influence on straw and seeds.

The particularly harmful effect of molybdenum on flowering, as reflected in seed production, was specially noteworthy in cucumber and Isleham Fen soils (Table 12). In cucumber soil the lower dressing was far more toxic to seed production than to the straw, but with the higher dose the damage was accentuated and seed production was almost nil. On Isleham Fen soil the point of extreme toxicity must have been reached with a much lower dose of molybdenum than was here used, as so little seed was produced even with the lower dressing.

The general trend of the molybdenum effect is again more easily seen by comparing the percentage dry weights in terms of the control for the different parts of the plant (Table 13). It may perhaps be permissible to demonstrate the general higher tolerance of flax straw to molybdenum poisoning by averaging and comparing the percentage figures for the eight soils. The lower dressing of molybdenum caused a mean drop to 77% in the straw, against 67% in the seeds, whereas the higher dose gave 71% in the straw, with a greater drop to 57% in the seeds.

TABLE 13. *Flax.* 1945. *Dry weights as percentage of controls. Mean of four plants*

		Straw		Seeds	
Soils	Control	Low Mo	High Mo	Low Mo	High Mo
Allotment soil	100	82·2	76·3	67·7	58·3
Allotment compost	100	93·9	89·4	88·7	80·7
Woburn soil	100	70·3	69·7	75·7	70·1
Woburn compost	100	85·1	79·7	99·3	79·0
Rothamsted Farm	100	91·2	97·2	92·2	84·0
Harpenden Common	100	105·9	99·8	92·1	86·1
Isleham Fen	100	50·0	42·3	2·1	0·7
Cucumber	100	38·0	13·8	21·3	0·1
Mean	100	77·1	71·0	67·4	57·4

1946 *experiment*

Seeds were sown on 21 March, about 5 weeks earlier than in the previous year. The seedlings were again allowed to establish themselves for 3 weeks before the first application of molybdenum, but the second was delayed for 50 instead of 20 days after the first treatment. These differences in dates of sowing and treatment may have been connected with certain variations in the results in the two seasons.

The development of the control plants was very similar in size and habit in all but the Isleham Fen soils, where they were much worse than in 1945 (Pl. 2, fig. 4). The addition of manganese sulphate to this soil caused a great improvement, but the plants remained much behind those in the other soils (Pl. 2, fig. 5).

In every soil some or all of the plants treated with the higher dose of molybdenum showed yellowing apices of the stem 2 days after the application of the poison, and in most plants it was noticeable, to a less degree, with the lower dressing. This yellowing was a transient phenomenon, and was not obvious after a few days except in the unthrifty Isleham Fen plants. A month after treatment the Woburn plants

had yellow cotyledons and some were beginning to fall over in the characteristic manner. By this time the Isleham Fen plants had become poor, thin and wiry, with the cotyledons yellow, and again a strong inclination to fall over, less marked where manganese was added. There was also a slight tendency to falling in the Littleport Fen soil, but this did not occur in all pots. By the beginning of June all the Woburn and Littleport Fen plants had turned bright yellow at the base of the stems, and the leaves were almost orange in the controls as well as in the treated plants. In the Waterbeach Fen soil the number of yellow plants increased with the molybdenum dressing, few occurring in the controls, but only one case of this yellowing was seen in the top-spit loam.

By this time the Isleham Fen plants were very small, with all their main stems dead. Laterals had developed, but were all chlorotic or were showing the characteristic yellowing, chiefly in the presence of molybdenum. Where manganese had been added the yellow and orange coloration had not appeared at all, even with the heavier treatment. Flowering began in early June, plants in Woburn and Littleport Fen soils being the first to bloom, the top-spit loam and compost being at least 4 days later, and the Isleham Fen soil with manganese a week later still. Before long the apices of the last-named plants became distorted in the treated pots, and hardly any flowers developed bolls, but the controls were normal in type, though smaller and weaker than in the other soils. At a very late date some poor flowers opened on the Isleham Fen plants without manganese, but none of these developed fruits and seeds in 1946, though a few were produced in 1945 on similar soil.

By the beginning of July there were no visible differences that could be attributed to the molybdenum treatment except on the Isleham Fen soil, but the plants in the 'straight' loam and Woburn soils were slightly riper than in the composts where peat had been added. The crops were retained until mid-August, when the straw and the great majority of bolls were fully ripe, and all were harvested at the same time. At harvest, after weighing and the removal and counting of the bolls, the individual straws were measured from the point of insertion of the cotyledons to the tip of the flowering shoot, the roots having been cut off at the cotyledon scar before weighing. The seeds, removed from the ripe bolls, were dried and weighed, but the straws from each pot were packed in bundles and sent to the Norfolk Flax Establishment for the determination of fibre content. There sample weights were determined, giving figures which could be applied to the original green weights to estimate the dry matter in the crop from each pot.

In 1946 the toxic effect of molybdenum was much less manifest than in 1945 (Table 14). The lower dose failed to reduce the dry weight of the crop in any soil except Isleham Fen with manganese. With the higher dressing there was a significant reduction in straw and seed in all soils except Waterbeach Fen and top-spit loam and its compost. There was even some suggestion of improvement with molybdenum in the straw on top-spit loam and the seed on Waterbeach Fen soil, but the amount of replication was too small to allow any weight to be placed on these results

without confirmation. On the Isleham Fen soil growth was much worse this year even on the controls, but again the lower dose of molybdenum was less harmful, though the proportionate effect of the heavier dressing was almost the same in both years. Curiously, the growth in 1946 with the addition of manganese to the Isleham Fen soil brought the control up to those of the straight soils in 1945, and in this case also the lower dose of molybdenum was extremely toxic, giving weights again corresponding closely to those in the 1945 undoctored soil.

TABLE 14. *Flax.* 1946. *Dry weights (g.). Mean of four plants*

O = control; L = low Mo; H = high Mo.

	Straw			Seeds			Total		
Soil	O	L	H	O	L	H	O	L	H
Top-spit loam	14·43	17·43	14·53	5·37	5·56	5·44	19·80	22·99	19·97
Top-spit compost	15·66	15·71	15·99	6·26	6·02	5·92	21·92	21·73	21·91
Woburn soil	14·20	14·35	12·68	4·06	4·11	2·89	18·26	18·46	15·57
Woburn compost	14·69	14·40	13·42	4·74	4·92	3·94	19·43	19·32	17·36
Isleham Fen	2·97	2·47	0·97	—	—	—	2·97	2·47	0·97
Isleham Fen + Mn	9·15	5·16	3·46	2·17	0·11	0·11	11·32	5·27	3·57
Littleport Fen	14·18	13·63	12·94	4·32	4·19	3·70	18·50	17·82	16·64
Waterbeach Fen	13·09	13·37	12·82	4·26	5·71	4·20	17·35	19·08	17·02
	S.E. 0·39			S.E. 0·14					

The diminished response to the poisonous action of molybdenum may have been due to the longer interval of time between the first and second treatments. This would allow more growth before the second dose was applied, and if the first dosage had been insufficient to cause real check to growth, there may not have been time for the extra molybdenum to act before vegetative growth was completed and the energies of the plant concentrated on seed production. On the other hand, as the seed weight was hardly affected by the lower dressing on most soils, it would also appear that some seasonal or environmental factor had been at work in 1946 which increased the resistance of the plants to molybdenum in all the tested soils except Isleham Fen. Here the addition of manganese was necessary for a small measure of seed production in the controls, but very little was formed with even the low dose of molybdenum. It might here be significant that the flowers did not appear until very much later than in any of the other soils, the intervals being at least a month and probably more. This different response in the two seasons is clearly seen by comparing Tables 13 and 15, giving the dry weights in percentages of those of the controls, providing yet another instance of the unpredictable way in which molybdenum acts even under conditions that are apparently similar.

The height of the flax was also not significantly affected by the molybdenum (Pl. 2, fig. 6) except in the Isleham Fen series. An interesting point, however, is that the composting of the rather heavy top-spit loam with peat caused an appreciable increase in height, both in the presence and absence of molybdenum, whereas the composting of the sandy Woburn soil caused a corresponding decrease.

TABLE 15. *Dry weight of straw and seeds expressed as percentage of controls. Mean of four plants*

O = control; L = low Mo; H = high Mo.

Soil	Straw			Seeds			Total		
	O	L	H	O	L	H	O	L	H
Top-spit loam	100	120·8	100·7	100	103·5	101·3	100	116·1	100·9
Top-spit compost	100	100·3	102·1	100	96·2	94·6	100	99·1	100·0
Woburn soil	100	101·1	89·3	100	101·2	71·2	100	101·1	85·3
Woburn compost	100	98·0	91·4	100	103·8	83·1	100	99·4	89·3
Isleham Fen	100	83·2	32·7	—	—	—	100	83·2	32·7
Isleham Fen + Mn	100	56·4	37·8	100	5·1	5·1	100	46·6	31·5
Littleport Fen	100	96·1	91·3	100	97·0	85·6	100	96·3	89·9
Waterbeach Fen	100	102·1	97·9	100	134·0	98·6	100	110·0	98·1

The inhibitory action of molybdenum on the production of ripe bolls and seed was specially well marked in the Isleham Fen series, but was also indicated in other cases by the persistence of green, unripe bolls at harvest. On the Woburn and top-spit loam, with and without peat, the green weight of the unripe bolls increased with the addition of molybdenum. With the heavier dressing the increase was very appreciable, but it was also strongly marked with the lighter dose in the presence of peat. Had these bolls developed and ripened they would have raised the seed production above that of the controls in the top-spit soil and compost, and as high as the controls in the Woburn series. Raising of seed production might also have occurred on the Littleport and Waterbeach Fen soils, but the inhibitory action of the manganese deficiency on the Isleham Fen soil prevented a similar response there.

Fibre determinations

The straw sent to the Norfolk Flax Establishment was treated by a method designed to minimize the number and duration of operations in order to reduce the risk of experimental error arising from loss of material. As the method used requires the full-length undamaged straw it was not possible to obtain the dry weights of the samples direct by oven drying, so the estimation was made by using one straw from each sample in a controlled scheme of bulking and calculating the dry weight of the whole from the results obtained. Percentage fibre contents were calculated from the dry weights of straw and fibre.

The straw from the Isleham Fen soil with and without molybdenum was too small and brittle for fibre extractions. The addition of manganese to the soil so improved the growth of the control plants without molybdenum that a reasonably high weight and percentage of fibre could be extracted, but the addition of molybdenum again caused the plants to be of the small and brittle type from which fibre could not be obtained. The quality of the fibre on the other soils did not show any striking differences except on the Waterbeach series, in which it was definitely finer and softer.

TABLE 16. *Flax fibre.* 1946. *Mean of four pots*

Soil	Weight of dry fibre (g.)				% fibre content in dry matter			
	Control	Low Mo	High Mo	Mean	Control	Low Mo	High Mo	Mean
Top-spit loam	3·01	3·18	2·79	2·99	20·8	18·3	19·2	19·4
Top-spit compost	3·42	3·24	3·32	3·33	21·8	20·6	20·8	21·0
Woburn soil	3·57	3·07	2·80	3·15	25·1	23·1	22·1	23·4
Woburn compost	3·32	2·96	2·63	2·97	22·6	20·6	19·6	20·9
Isleham Fen	—	—	—	—	—	—	—	—
Isleham Fen + Mn	1·58	—	—	0·53	17·3	—	—	(17·3)
Littleport Fen	3·17	3·03	2·89	3·02	22·2	22·2	22·3	22·2
Waterbeach Fen	3·13	3·10	3·02	3·08	23·4	23·2	23·5	23·4
	S.E. treatment means 0·37				S.E. treatment means 1·01			
	S.E. soils means 0·07				S.E. soils means 0·58			

Excluding the Isleham Fen soil, with its low output, the mean weights of the fibre from the different soils were almost the same (Table 16). No significant difference in the weight of fibre was associated with molybdenum treatment.

Although the weight of fibre was in general little affected by soil or treatment, the percentage of fibre in the dry matter was influenced to some extent by both these factors. Apart from the Isleham Fen soil, the lowest percentage of fibre occurred on the top-spit loam, possibly improved in the compost. The Woburn soil output was relatively high, but this was significantly reduced by composting. High output was also obtained on the other two Fen soils, the Waterbeach Fen giving slightly the better results.

DISCUSSION

These experiments with molybdenum carried out since 1942 with different soils and crops under controlled conditions show the great variability of response to the toxic effect of the element. This falls into line with the results of other workers who have reported widely different responses from various parts of the world, differences that appear to be associated with soils, crops, seasons and climatic conditions. The inconsistency of the findings is still a troublesome problem to investigators, and even yet no satisfactory explanation is forthcoming.

The results of the five years' work at Rothamsted have been epitomized in Table 17, giving an overall review of the position.

Of the three crops grown, tomatoes are in general the most resistant to the harmful action of molybdenum though the response differs to some extent with variety. In ordinary loam, either as a straight soil or composted with leaves or peat, the poisonous effect was rarely seen, as Kondine Red only once showed a significant reduction of crop, in 1943, when sodium molybdate was given in two doses. Potentate is apparently rather more susceptible, as in 1944 the crop was reduced by a lower rate of molybdate, which had no effect on a parallel series of Kondine Red. In other years, however, there was no effect in either variety with the same soils and

TABLE 17. *Summary of Rothamsted trials* 1942–6

	Tomatoes (1942–1946 experiments, see Tables 1 & 2)	*Solanum*	Flax
		1945 experiment	
Woburn soil	High, toxic	Low, no effect High, almost killed	Reduction, both doses
Woburn compost	No toxic effect	No toxic effect, or High, almost killed	Progressive reduction, but less than in soil
Allotment soil	No toxic effect	Reduction with both doses	Reduction with both doses
Allotment compost	No toxic effect	No toxic effect, or High, almost killed	Slight reduction with both doses
Farm soil	No toxic effect	Progressively toxic with dose	No toxic effect
Common soil	No toxic effect	Both doses slightly toxic	No toxic effect
Isleham Fen	Low, no toxic effect High, killed	Heavy reduction with both doses	Progressive toxicity with both doses
Cucumber soil	No toxic effect	Killed with both dressings	Low very toxic High, more so
		1946 experiment	
Woburn soil	Low, very toxic High, killed	Low, no effect High, heavy reduction	Low, no effect High, slight reduction
Woburn compost	Low, no effect High, bad damage	Little reduction	Low, no effect High, slight reduction
Top-spit loam	No toxic effect	Reduction with both doses	No toxic effect
Top-spit compost	No toxic effect	Reduction with both doses	No toxic effect
Isleham Fen	Steady reduction with dose of Mo	Low, heavy reduction High, almost killed	Progressive reduction in presence of Mn
Littleport Fen	No effect	—	High, slightly toxic
Waterbeach Fen	No effect	—	No toxic effect

varying molybdate, except once with a very heavy dressing with Potentate in compost.

A comparison between soils of very different natures confirmed the general resistance of tomatoes to molybdenum poisoning except on the sandy Woburn soil and the manganese-deficient Isleham Fen soil. Even here, however, inconsistency showed, for whereas in 1945 the stronger dose of molybdenum killed the plants in the Fen soil, allowing about half a crop to develop in the Woburn, in 1946 the results were reversed, the molybdenum proving more toxic in the Woburn than in the Fen soil. The lack of toxic action on tomato in cucumber soil was noteworthy, as in this soil both molybdenum dressings were very poisonous to flax and were fatal to *Solanum*.

Broadly speaking, *S. nodiflorum* was the most sensitive of the three crops to poisoning by molybdenum, the degree of crop reduction being greater than in flax except in some plants in the two composts. Here the low dose proved harmless, but

a differentiation occurred with the high dressing, some plants being unhurt, while others were almost killed, exemplifying the individual response to what seem to be borderline concentrations.

Again the toxic action of molybdenum was more marked in Woburn and Fen soils than in the others. A point of special interest in this connexion is that though tomatoes are much more resistant than *Solanum* and flax in most soils, they were the most seriously affected in Woburn soil, even the lower dose of molybdate being very toxic. At this strength in this soil *Solanum* was unaffected, but with the higher dose which killed tomatoes it was heavily poisoned, flax escaping more lightly. Flax was also unaffected in Harpenden Common soil, and in top-spit loam and its compost, *Solanum* being somewhat poisoned in both.

Repeated experiments have therefore confirmed that in the present state of our knowledge it is impossible to forecast how any crop will respond in any soil to treatment with varying doses of molybdenum. The key to the problem may depend on a number of factors, which in field tests and even under experimental conditions in pots may be outside the control of the investigator.

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EXPLANATION OF PLATE 2

Left to right in each figure:
Control; Low molybdenum; High molybdenum

Fig. 1. *Solanum nodiflorum* in old cucumber soil.
Fig. 2. *Solanum nodiflorum* in Isleham Fen soil.
Fig. 3. *Solanum nodiflorum* in allotment soil.
Fig. 4. Flax in Isleham Fen soil.
Fig. 5. Flax in Isleham Fen soil + manganese sulphate.
Fig. 6. Flax in Waterbeach Fen soil.

(*Received* 11 *July* 1947)

BRENCHLEY—*Toxic action of molybdenum in relation to soils and crops*

PROBLEMS IN THE CULTIVATION OF WINTER LETTUCE

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In a four-year study of winter lettuce cultivation, attention has been given to the effects of variety, dates of sowing and transplanting, method of preparing seed-beds, nature of field soil, and fungicidal treatments upon percentage survival and date of maturity of the crop.

The inferiority of the variety Trocadero Improved to such a winter variety as Imperial is due mainly to its susceptibility to *Pythium* root rot. This disease often causes severe thinning of the stand of Trocadero seedlings and dwarfing of many of the survivors.

Late autumn plantings of Imperial and other winter varieties survived better overwinter on a medium-heavy loam than on a light sandy soil. The disparity varied in different seasons and was not associated with severity of winter.

The dates of maturity of the crop in relation to dates of sowing and transplanting have been determined over the four-year period. On the basis of these, and on the records of survival and of market prices, an estimate can be obtained of the financial aspects of different cultural methods.

Fungicidal treatment in the seed-bed of plants set out in the field in November gave little improvement in survival; but pronounced benefit was obtained by February or March dusting of seedlings which had overwintered in the seed-bed.

The onset of disease in overwintering seed-beds was found to be influenced by the nature of the soil and by the manner of preparation of the seed-bed.

The interrelations of overwintering and soil type are briefly discussed, with special reference to the effects of soil type (i) on the root system, and (ii) on microbiological interference with invasion of the stem by *Botrytis*.

Introduction

An earlier paper (Smieton & Brown, 1940) described experiments on the cultivation of 'spring lettuce', i.e. of lettuce sown in cold frames at about the end of October and transplanted to the open field in early spring. The main disease problems were outlined with some assessment of the importance of each to the grower. Apart from mildew, which may cause considerable damage in very mild humid winters, the chief fungal diseases were damping-off, caused by species of *Pythium* and by *Rhizoctonia Solani*, the latter being the more important, and the 'red-leg' disease caused by *Botrytis cinerea*. Application of a dust containing pentachloronitrobenzene gave a good control both of damping-off caused by *Rhizoctonia* (but not that caused by *Pythium* spp.) and of *Botrytis* disease.

From 1939 onwards, attention was directed to the parallel problems of 'winter lettuce', i.e. of lettuce which is sown out of doors, either in seed-beds or where the plants are intended to mature, and which is grown throughout under open-field conditions. It is known that such lettuce is subject to the same fungal diseases as lettuce overwintered in cold frames, and that in addition the 'ring-spot' (sometimes

called 'rust') disease, caused by *Marssonina pannattoniana*, may in some seasons produce devastating losses. The relevant information is derived from somewhat casual reports, relating to different districts, soils, methods of cultivation, varieties, etc., and so is of little use in determining the factors responsible for the varying severity of disease.

It was planned, therefore, to cultivate winter lettuce on a fairly substantial scale over a number of seasons and to record systematically the history of the crops. After some preliminary work in 1939–40 and 1940–1, a general plan, to be outlined below, was adopted and adhered to over the four seasons 1942–6. The work is in essence a survey of the problem, in which the prevailing conditions are fairly well defined. As such, it is of necessity incomplete, as it covers only a limited range of climatic conditions, soil types, varieties, etc. Certain results have already appeared, but as will be shown later there is much that still requires elucidation. A main object of this investigation was to define the problems as closely as possible, with a view to a more detailed examination later. Though the work is thus still in progress it is hoped that publication at this stage may have the effect of stimulating similar investigations elsewhere, so that in due course a more general picture may be forthcoming.

Experimental method

(*a*) *General plan.* This was to sow lettuce in seed-beds at several dates, to transplant to the open ground, into a variety of soils, again at several dates, and to record in due course the losses which took place. Certain fungicidal treatments were included in the programme. Cutting of the crop was done systematically, when the plants had reached a certain stage of hearting. The frequency of cutting varied somewhat with the weather, but was at least twice per week. As there is always a proportion of plants which are slow in hearting or which do not heart at all (on account of mosaic disease, or of fasciation, or of damage to the terminal bud leading to a diffuse leafy type of plant by growth of side-buds), the date when three-quarters of the plants of any batch had been cut was taken as the date of maturity. The produce was sold in the open market and a record of prices obtained. It was thus possible to secure, for each method of treatment, an estimate of the number of plants which grew to maturity, of the date at which they matured and of their market price, so that the relative economic value of different treatments could be assessed.

The general plan was modified slightly from year to year, according to circumstances, but may be exemplified by the main sowings of 1944–5.

Four seed-beds (each of about 10×4 yd.) were sown on 8 September, in drills 1 ft. apart, with the varieties Imperial and Trocadero Improved at a standard rate of 0·25 g. seed per yard run. The plants of this sowing were all removed in early winter (end of October to middle of November), by which time they were of a size convenient for handling, and placed in their final positions.

A second sowing of the same varieties was made in the same seed-beds on 18 September, the drills alternating with those of the earlier one. A small proportion of these plants was set out in the field at the same time as those of the first sowing, but the majority were left in the seed-bed until the end of February. The following comparisons were thus available in a replicated experiment, all the seedlings being derived from the same seed-bed: (*a*) sown 8 September, transplanted November; (*b*) sown 18 September, transplanted November; (*c*) sown 18 September, transplanted February.

In addition, sowings of the variety Trocadero were made in cold frames in the usual manner, these ranging over the period 15 October to 7 November. The seedlings were planted out in February–March as they became available. A comparison was thus made of the performance of Trocadero as a winter and as a spring lettuce.

As the work proceeded it became clear that the plants derived from the several seed-beds, especially when allowed to stand there over winter, varied considerably in health at the time of transplanting, and consequently also in their ability to survive in the field. For the last two years, therefore, additional seed-beds, in different sites and prepared in different ways, were set up and their behaviour recorded. This part of the work will be dealt with in a later section.

(*b*) *Planting-out ground.* The plots at Slough used for this work have been liberally fertilized each year with farmyard and artificial manures, together with dressings of lime. Their fertility is indicated by the size of the lettuces produced in the years with good spring rainfall. The experimental conditions were therefore of the type aimed at in intensive market gardening. From June to October the ground was cropped chiefly with tomatoes and potatoes.

The area of land available for vegetable cultivation is only about 2 acres, but offers a variety of soil conditions. At one end is a patch of low-lying peaty soil over clay, which is subject to waterlogging in winter. Earlier experiments gave very low survival of plants on this soil, so subsequent tests were confined to the higher land. This ranges from a well-drained, light, gravelly soil at one end to a good, medium loam at the other. The expectation was that, as losses of winter lettuce are mainly caused by *Botrytis cinerea*, overwintering would be best on the lighter soils; one of the main conclusions from this work is that precisely the opposite occurs.

Planting was done, as far as possible, by the same workman. The unit plot was a row of from twenty to fifty plants, according to the dimensions of the particular piece of ground. A fourfold or fivefold replication was usually arranged. As planting dates were in November or in February–March when days are short and weather and state of ground unpredictable, and as it was important that each experimental planting (usually involving a replicated lay-out in several plots) should be completed in one day, a time-consuming strict randomization of the unit rows was not attempted. The arrangement was typically as follows: *a*, *b*, *c*; *a*, *b*, *c*, and so on.

The seedlings for any one experiment were lifted in the morning, sorted for size

and quality, the criterion for the latter being the presence or absence of lesions on the hypocotyl at the lower leaf bases; then those of a certain grade were well mixed and planted out later on the same day. Approximately 30,000 plants were set out in each year. Ordinary commercial spacing, i.e. 1 ft. × 9 in., was adopted.

Counts of surviving plants were made at two dates: first, when the plants had begun to grow rapidly in the early spring, i.e. about mid-March for plants set out in November and about 3–4 weeks after setting out for spring plantings. The second count was made when the cutting of the particular experimental batch began, which was from about the end of April onwards. The date when three-quarters of the plants recorded at the second count had been cut was taken as the date of maturity of that particular batch.

Causes of losses of plants

The part played by the various agencies causing losses will now be briefly indicated.

(*a*) *Cutworms*. A close watch was kept on each experimental planting, especially in its early stages, for cutworms, and these were destroyed as found. Losses from this cause were slight.

(*b*) *Wireworms*. Some plots, on ground recently prepared from grassland, were ruined by wireworms. Later these disappeared and no further damage of this kind occurred.

(*c*) *Slugs*. These were troublesome, and although systematic poisoning with metaldehyde was adopted, it did not completely eliminate this source of loss. Survival of plants was best on the heavy ground *in spite of* greater liability there to slug damage.

(*d*) *Aphides*. These occurred each year and sometimes rather abundantly. Apart from transmitting virus disease they interfere with the hearting of lettuce plants, at least when present in large numbers, and even moderate numbers of aphides may reduce market value. No attempt has been made at control, but the experience obtained has clearly shown the importance of aphis infestation of winter lettuce and the need for its further investigation.

(*e*) *Marssonina*. As an outbreak of *Marssonina* disease would have vitiated these experiments great pains were taken to exclude this fungus by raising disease-free seed of the three varieties chiefly used, Imperial, Majestic and Trocadero Improved. No difficulty has been experienced in obtaining satisfactory yields of Imperial and Majestic seed, but Trocadero seed crops have usually failed. Most of the sowings of the last named have therefore been from commercial seed. In the outcome the experimental plantings have not always been completely clear of *Marssonina*. Occasional infected plants or patches of these have occurred in the seed-beds, and have been promptly cut out, along with a further 6 in. on both sides in the drill. The chief source of contamination is probably the bought Trocadero seed. It may be noted that the most drastic test of the freedom of a batch of home-grown seed from contamination with *Marssonina* is to examine the self-sown seedlings which come up on the bed where the seed has been produced. Germinating in the presence

of the trash of the mother plants, the seedlings run the maximum risk of infection. If they remain uninfected there is a strong presumption that the mother plants, and therefore the seed itself, are free of the disease. Only in the last batch of Imperial seed, saved in 1945, does this complete freedom from contamination appear to have been attained. Nevertheless, by the precautions outlined above, *Marssonina* disease has been kept at negligible proportions.

(*f*) *Botrytis*. So far as can be seen this is the most important killing agent of winter lettuce plants set out in the field. Damping-off disease, due to *Rhizoctonia Solani* and *Pythium* spp., occurs in the seed-beds, and root damage associated with *Pythium* spp. and leading to dwarfing of the plants, especially with the variety Trocadero, may be found at all times throughout the growth period. It is for further investigation to determine whether attack by these latter organisms promotes the later invasion of the plants by *Botrytis*. Just as with seedlings grown in cold frames, it is difficult to state where *Rhizoctonia* attack finishes and *Botrytis* attack begins. In the early stages of the plant, when cotyledons and leaves are still intact, it is easy to diagnose *Rhizoctonia* disease, especially when invasion has taken place well below the insertion of the cotyledons. Later on, the predominant fungus, both to the naked eye and on the isolation plate, is *Botrytis* which has entered by way of the dying leaf bases, but one cannot exclude the possibility that *Rhizoctonia* may also have played a part. One can say, however, that *Botrytis*, whether it attacks primarily or secondarily, is the all-prevailing fungus which determines what the stand of winter lettuce will ultimately be. It is noteworthy that the related fungus, *Sclerotinia libertiana*, which so freely causes 'lettuce-drop' in America, has only been seen once in the course of all this work.

Except for certain plantings in low-lying ground, which succumbed steadily throughout the winter, losses in a crop of a typical winter variety like Imperial do not show up until growth recommences in the spring. With the variety Trocadero, which is not classed as a winter lettuce, conditions are somewhat different, as will be pointed out later. It is clear that death of the plants is not due to cold winter conditions as such, for even the unusually severe frosts of 1939–40 (and of 1946–7) did not kill the plants outright. As growth recommences in the early spring, fungal invasion becomes active, and within a few weeks the bulk of the casualties takes place. Thereafter the loss of plants is much more gradual, but continues slowly up to the time of maturity and later still if the plants are kept for the production of seed.

(*g*) *Miscellaneous causes*. These have already been alluded to in connexion with the residue of unmarketable non-hearted plants which remain after the bulk of the crop has been cut. Mosaic is the most important cause of loss, plants infected early being small, mottled and unhearted, and those infected later, if they make a heart at all, having a sickly yellow appearance. The percentage of such plants has been usually under 5%, but in 1945–6 it was higher, especially in Trocadero, in which it ranged between 10 and 20%. The disease is claimed to be carried in a small percentage of the seed, but it is noteworthy that out of 1442 seedlings raised on a

segregated plot from mother plants all of which definitely had mosaic, not one showed symptoms of disease. This was at a time when occasional seedlings of the same age and with well-marked symptoms of mosaic disease could be found elsewhere in the experimental grounds. In the writers' opinion, mosaic of winter lettuce is more likely to derive from aphides which have migrated to the young plants in autumn from late summer crops, which are usually abundantly infected.

Effect of planting-out ground

The first indication that seedlings survived better in heavy than in light ground was obtained in 1940–1, when a batch of Imperial sown on 6 September and transplanted on 7 November gave 60·1 and 21·8% survival in the heavier and lighter ends respectively of the same field. From 1942 onwards this effect was systematically tested by planting batches of seedlings, taken from the same seed-bed, on light and on heavy soils. The relevant data are assembled in Table 1.

The average survival in parallel tests on plots D (heavy) and B (light) over four seasons is shown in Table 2.

Of the twenty-one comparisons which were in themselves significant, eighteen were significant well above the 100 : 1 level. Apart therefore from one year (1943–4) the differences were very distinct, and in 1945–6 the performance of plot B was extremely bad, in which year survival on D had also fallen off somewhat. The records of rainfall and temperature over the months October–April in each season have not afforded any clue to the cause of the seasonal fluctuations. The best overall survival was in 1943–4 and the worst in 1945–6. The mildest winter was 1942–3 and the severest 1944–5. In no year was there appreciable snowfall which might have acted as a protection. Though it may be assumed that conditions of temperature and moisture largely determine percentage survival, the mode of action of these factors cannot as yet be stated with precision.

It might be suggested that the figures of Table 2 (from 1943–4 onwards) indicate the development of fungal 'sickness' in the plots, especially in B. An increase of *Botrytis sclerotia* in soil repeatedly cropped with lettuce is possible, but an independent source of inoculum is practically always available in the conidia on moribund plant material of many sorts. With *Rhizoctonia Solani* a build-up is known to occur, especially in seed-beds or frames which are used for lettuce year after year, and as attack by *Rhizoctonia* may lead to *Botrytis* attack, the latter might thus be indirectly intensified by repeated cropping. The bulk of the evidence, however, is against such a view. Thus, the marked fall in survival on plot B from 1943–4 to 1945–6 was arrested in 1946–7. Though the experiments were carried out on a reduced scale in that year, survival on plot B was again in the neighbourhood of 80% in spite of an unusually severe winter.*

* The corresponding batches on D were destroyed by an epidemic of slug damage, caused presumably by the unusually wet summer. A comparison between winter survivals on B and D was thus not possible.

TABLE 1

Year	Variety	Date of sowing	Date of planting	Survival (to nearest 0·5 %) Heavy plots D	E	F	Light plots B	P	S
1942–3	Imperial	10 Sept.	23 Oct.	92	—	88	81	—	43
	,,	,,	24 Oct.	90	—	—	—	—	65·5
	,,	,,	25 Oct.	90·5	—	91·5	85·5	—	—
	,,	,,	30 Oct.	80·5	—	—	65	—	—
	,,	,,	,,	85·5	—	—	67·5	—	—
	,,	,,	31 Oct.	88	—	—	65	—	—
	,,	,,	,,	90	—	—	72·5	—	—
1943–4	,,	14 Sept.	9 Nov.	94	84	—	86	63	48
	,,	,,	10 Nov.	91	80·5	—	87·5	63	52
	Majestic	,,	,,	82	82·5	—	82·5	75	53
	Imperial	,,	,,	92·5	85	—	82	—	53
	,,	,,	11 Nov.	76	84	—	73·5	—	—
	,,	,,	12 Nov.	75	—	—	76	—	—
1944–5	,,	8 Sept.	7 Nov.	78	86·5	—	64	—	—
	,,	,,	,,	74·5	84	—	43·5	—	—
	,,	,,	,,	78	83	—	57·5	—	—
	,,	,,	,,	74·5	86	—	54	—	—
	,,	Self-sown (Aug.)	13 Nov.	80	92	—	61	—	35
	,,	8 Sept.	,,	84	85·5	—	46	—	—
	,,	18 Sept.	,,	87	80·5	—	61	—	—
	,,	9 Sept.	15 Nov.	—	89	—	46·5	37	—
	,,	,,	,,	—	82	—	48	36	—
1945–6	,,	10 Sept.	7–8 Nov.	68	71	—	11	—	—
	Majestic	,,	,,	73	78·5	—	19·5	—	—
	Trocadero	,,	,,	70·5	69	—	17	—	—
	Imperial	21 Sept.	,,	69	63	—	27	—	—
	Majestic	,,	,,	66·5	73	—	32	—	—
	Trocadero	,,	,,	53·5	43	—	31	—	—
	Imperial	10 Sept.	9 Nov.	71·5	76	—	11·5	48·5	—
	Majestic	,,	,,	66	76	—	11	49·5	—
	Trocadero	,,	,,	63·5	49·5	—	13	39	—

TABLE 2

Year	No. of tests	% survival D	B	D > B	No. of significant results ($P > 20:1$)
1942–3	6	87·8	72·8	6	5
1943–4	6	85	81·3	4	1
1944–5	7	80·9	53·5	7	6
1945–6	9	69	18·7	9	9

Further evidence is to be obtained by noting the earlier history of some of the plots. In the three years previous to 1942, three lettuce crops had been grown on parts of the E plot, none on other parts of the same, and none on D. From 1942 cropping has been the same in both. Nevertheless, survival has been fairly uniform on the various parts of E and has not differed materially from that on D. There is

thus no suggestion of an association of more intensive cropping with reduced survival on these plots. On the other hand, the behaviour of certain plots on light land could be interpreted on this view. Thus in 1943–4 (see Table 1) plot B gave better survival than plots P and S; in 1945–6, B was inferior to P. In the years before the experiments recorded here, P and S had carried lettuce crops repeatedly, but less often and on a smaller scale since 1942; the converse applies to B. It might be suggested therefore that plots P and S were already 'fungus-sick' in 1942 and had remained in much the same condition since then, whereas B had developed sickness by annual cropping in the years of these experiments.

In the writers' view the evidence that any form of fungal sickness has developed on the field plots where the plants have been set out to mature is as yet insufficient. Further discussion of factors bearing on winter survival of lettuce will be given in the last section of this paper.

Effect of dates of sowing and planting on survival and date of maturity

The main planting of Imperial in 1944–5 will serve as an illustration. The particulars were as follows:

(1) Seed sown on 8 September; transplanted 13 November.
(2) Seed sown on 18 September; transplanted 13 November.
(3) Seed sown on 18 September; transplanted 26 February.

Each lot was set out in a fourfold replication on plots B, D and E in rows of forty plants on B, and of fifty plants on D and E. Furthermore, the seedlings were taken separately from three seed-beds, and as these did not behave differently, there was in effect a 12-fold replication. At the November planting, the rows to be planted in the spring were left blank, to be filled up later. Thus, while the November plantings were made into recently dug ground, those in spring were made into ground which had become consolidated over winter. As this difference might of itself affect the growth and survival of the plants, two large-scale subsidiary experiments were carried out. These showed that, both for late autumn and for early spring planting, the state of consolidation of the ground had no effect, within wide limits, upon the survival and rate of growth of the plants.

Table 3. *Variety Imperial*

	Sown 8 Sept., planted 13 Nov.		Sown 18 Sept., planted 13 Nov.		Sown 18 Sept., planted 26 Feb.	
Plot	% survival	Date of maturity	% survival	Date of maturity	% survival	Date of maturity
B (light)	45·8	26 April	60·9	28 April	46·1	11 May
D (heavy)	83·9	21 ,,	86·8	27 ,,	53·5	9 ,,
E (heavy)	85·5	21 ,,	80·5	26 ,,	51·7	15 ,,

Table 3 gives the summarized results for seedlings from one seed-bed. As before,

the date of maturity is the day on which 75% of the plants present when cutting began had been cleared.

The overall result of this experiment, when the two other sets of replicates not shown in the table are considered, was as follows:

(*a*) *Percentage survival:* approximately 80% in both the November plantings on plots D and E; approximately 50% for the same on plot B. The spring plantings on all gave a survival of about 50%.

(*b*) *Date of maturity:* no constant difference between plots B, D and E.

(i) Earlier sowing with November plantings gave 5 days earlier maturity than later sowing with November planting.

(ii) Later sowing with November planting gave 13 days earlier maturity than later sowing with February planting.

The above experiment has been repeated over four seasons, and the summarized results will now be stated.

(*a*) *Percentage survival.* Whereas in the experiment just quoted the winter (November) plantings from the later sowing survived as well as those from the earlier sowing, the general rule is that they do not do so, especially on the heavier ground. In the following list, each pair of figures relates to a planting on the same date, the first figure from an earlier sowing and the second from a sowing approximately 10 days later. The comparative figures are: 80·5, 85·5; 75·8, 62·0; 84·3, 63·0; 68·2, 69·2; 70·8, 63·2; 73·0, 66·7; 78·4, 73·2; 70·7, 55·5; 69·2, 43·2.

Of these nine comparisons all but two are in favour of the earlier sowing. The difference is probably due to the fact that plants from the later sowing are usually rather small at the time of transplanting. They are thus more likely to be loosened by frosts and by earthworms which pull at the withered lower leaves. On the average, plants required for transplanting in November should not be sown in outdoor seed-beds later than the second week of September.

The survival of lettuce transplanted from outdoor seed-beds in early spring (February–March) is extremely variable, ranging from just over zero to almost 100%. The condition of the seed-bed at transplanting time is largely responsible for this variation, and will be considered more fully later.

(*b*) *Date of maturity.* As would be expected, this varies from year to year according to the weather, both absolutely, and relatively as between the different treatments.

Imperial. Plants from the first sowing (*c.* 10 September) which are transplanted in early winter (beginning to middle of November) are the first to mature. Those of the second sowing (*c.* 20 September) which are transplanted at the same time, mature a few days later. In nine such comparisons extending over four seasons, the average difference has been 3–4 days (min. 0; max. 5). Plants of the second sowing which are not transplanted until early spring (middle to end of February) are later still in maturing. Thus as compared with early winter planting from the same seed-beds, the average retardation in thirteen comparisons extending over six

seasons was about 7 days. In 1944–5 it was 13 days, in 1943–4 it was 2–4 days. The general position may thus be summarized as follows:

Early sowing followed by early planting gives 3–4 days advance in date of maturity over late sowing and early planting, and 10–11 days advance over late sowing and late planting.

Majestic. In the comparatively few tests carried out with this variety, it has behaved similarly to Imperial.

Trocadero. Repeated tests with this variety have emphasized that it is not a satisfactory winter lettuce; hence data for Trocadero are omitted. Its survival, either in seed-bed or when planted out, is almost always inferior to that of Imperial, and many of the plants which do survive are dwarfed. This dwarfing is associated with fungal invasion and destruction of the roots which may be fatal or result in an undersized plant which develops new roots higher up the stem as the spring advances. Outwintered Trocadero therefore matures late and over a longish period, and the date of maturity of such stands may actually be later than that of plants grown in cold frames and transplanted to the open ground in February–March.

Comparison of varieties

The varieties Imperial, Majestic (a strain of May King), Arctic, Improved Unrivalled and Trocadero Improved have been compared as winter lettuce with respect to survival, date of maturity and other desirable features. The unsuitability of Trocadero has already been noted. Improved Unrivalled was rejected after 2 years' trials because it showed inferior survival and hearted late and unevenly. There was no constant difference in survival between the remaining three, but Arctic was rejected after 2 years as the mature plants were too small. Majestic matures a few days earlier than Imperial, and in a favourable spring produces a solid heart of good size, but in a dry spring many of the plants are not large enough. Imperial has the demerit that late-maturing plants do not heart properly; the heads 'turn in' and begin to fill up, but soon open out again and the plants run to seed. This is presumably a photoperiodic effect, to which Majestic (like Trocadero) is less prone. It would be worth while, therefore, to explore the possibilities of various strains of the May King type for use as winter lettuce.

An attempt was made to select a strain of Imperial to give enhanced overwinter survival. Seed was taken from ten strong plants which had stood up in a plot where nearly the whole stand had died off, but this proved to be no more resistant than the original stock.

Fungicidal treatments

Apart from one experiment in which certain soil-sterilizing chemicals were applied to a plot on which winter lettuce had always overwintered badly, the treatments took the form of spraying or dusting the seedlings while still in the seed-bed.

(1) *Soil treatment*

A piece of ground (plot S, of light texture) was divided into twenty equal plots of approximately 5 sq.yd. each, over which the following treatments were randomized:

(*a*) Untreated.

(*b*) Watered with 'Uspulun' (active principle, mercury chlorophenolate) at the rate of 2 oz. in 5 gal. of water per plot.

(*c*) Watered with formalin, 1·5 pints in 19·5 gal. water per plot; treated ground then covered with sacks for 10 days.

(*d*) Top-dressed with 2·3 oz. Folosan (pentachloronitrobenzene) dust per plot; dressing lightly raked into upper soil layers.

Later the ground was uniformly planted with Imperial seedlings, sixty-three per plot. The final survival figures were: Control, 33%; Uspulun, 35·8%; Formalin, 35%; Folosan, 34·4%.

There was obviously no improvement by the treatments, and therefore no evidence of the presence of soil sickness (cp. p. 166).

(2) *Seedling treatment*

Treatments at approximately monthly intervals (viz. in December, January and February) have been given to drills in outdoor seed-beds of Imperial and Trocadero, as follows:

(*a*) Bordeaux Mixture (2 : 10 : 40), applied as a spray.

(*b*) Shirlan (1·5% dilution), applied as a spray.

(*c*) Folosan, applied as a dust.

These emphasized the value of the last named, in spite of some phytocidal effect, but also gave the unexpected result that the sprayings with Bordeaux Mixture were actually harmful. Two such replicated experiments with Imperial gave the results shown in Table 4.

TABLE 4. *Percentage survival*

	Exp. 1	Exp. 2
Control	28·5	82
Bordeaux Mixture	17	49
Shirlan	40	78
Folosan	81·5	94

Exps. 1 and 2 were carried out with plants which gave respectively poor and good survival in the controls. The outstanding features are the beneficial effect of Folosan in Exp. 1 and the depressing effect of Bordeaux Mixture in Exp. 2.

The plants treated with Folosan showed most leaf scorching (from the combined action of frosts and fungicide); those treated with Bordeaux Mixture and with Shirlan were indistinguishable from the controls. There was thus no relation between visible phytocidal effect and capacity of the seedlings to survive. Further, although laboratory tests by the usual glass-slide technique show that Bordeaux

Mixture and Shirlan both surpass Folosan in their inhibiting effect on the germination of *Botrytis* spores, nevertheless, when applied to the plants, Folosan far exceeds the others in protective value. The explanation of this apparent discrepancy requires further research.

In the majority of experiments with fungicides, treatment was applied once only, viz. just before the plants were taken up for transplanting. The only substance used has been Folosan, chiefly in the form marketed before the war, but in the last 2 years also as the tetrachloronitrobenzene preparation called Folosan (D.B. 905).

With seedlings transplanted in November, a single dusting at that time has very little effect upon the final survival figures. The treated plants generally show a better stand in early spring, but by the time cutting begins, the difference has largely if not entirely disappeared. On the other hand, a single treatment applied to plants which have overwintered in the seed-bed and which are transplanted in February or March has produced very beneficial results. The percentage survivals in nineteen such comparisons over 5 years were as follows, the figures for the controls being first in each case:

Imperial: 37·5, 77; 33·3, 86; 74, 90; 98·8, 98·5; 63·2, 80; 76, 81·5; 39·5, 63; 56·3, 89·4; 48, 86; 70·8, 78·3; 12·3, 53·8; 53, 59; 66·7, 77·3; 71·5, 85·1.

Trocadero: 74·8, 91; 68·3, 78·7; 75·5, 90·5; 70, 86·8.

Majestic: 42, 66.

In all these experiments (and in fact throughout the work recorded in this paper) only seedlings which showed no obvious lesions* on the stem were used. Nevertheless, the survival of undusted plants in the field may be very low, e.g. 12·3% in one of the above tests. On the other hand, a nearly perfect stand (98·8%) was obtained with plants from an exceptionally good seed-bed. The varied behaviour of seed-beds will be dealt with later.

The above figures show that the degree of improvement effected by a single dusting at the time of transplanting has varied considerably. As the plants normally were crowded in the rows, and their stems typically clothed with leaf bases, dusting was vigorous to ensure penetration into the mass. Such overwintered plants show considerable resistance to phytocidal damage. In all these tests, with dust added to excess, the maximum check to maturity was 3 days.

In seasons 1944–5 and 1945–6, the new form of Folosan (which will be referred to as D.B.) has been tested against the old form, both with frame-grown and outdoor lettuce seedlings. Folosan contains 20% pentachloronitrobenzene as active constituent on talc filler, and Folosan D.B. 905 contains 5% tetrachloronitrobenzene on a china-clay filler. A straight comparison of the two active principles has not yet

* Lettuce seedlings which have stood overwinter out of doors have already lost a number of the lower leaves, so that the stems are invested with more or less decayed leaf bases. By stripping these off one could detect lesions on the stem which would otherwise escape notice. It does not follow that plants with small lesions or discolorations on the surface of the stem will necessarily prove to be casualties. In grading seedlings the only practical method is the one which the careful grower would adopt, viz. to discard any seedlings which show an obvious lesion in the region of the leaf base.

been attempted, the tests being merely of one dust against another, with each applied heavily. D.B. has usually given the better results with seedlings in frames, and Folosan with outdoor lettuce. The former has the great advantage that it can be applied to seedlings in frames, without causing a visible check, and it may safely be used while the seedlings are very small (e.g. in November) in order to prevent damping-off by *Rhizoctonia Solani*. For that purpose, Folosan had to be used very sparingly, otherwise a material check to growth was administered.

The results obtained in these experiments with frame-reared seedlings follow the same lines as those already reported (Smieton & Brown, 1940) and need not be repeated here. One interesting addition is the following. In a comparison between undusted and thrice Folosan-dusted frames (var. Trocadero) the respective percentages of survival were 38·2 and 81·2. At the date of this planting (18 March) batches of each sort were packed in tins and held at 10° C. for 7 days. When again examined, it was obvious that the control seedlings had considerably deteriorated, as shown by the following grading:

	Apparently sound	With obvious lesions
Control	100	57
Dusted	156	0

Plantings were then made of the apparently sound plants and these gave the following results: control, 11% survival; dusted, 80%.

A similar experiment with D.B. dust gave the same result. An additional use of these dusts is thus indicated, viz. as preservatives when lettuce seedlings are sent over a distance or when, after the plants have been lifted and bulked, planting in the field is held up, e.g. by severe night frosts or by rainy weather.

Table 5 illustrates the value of Folosan and D.B. in improving the stand in an outdoor seed-bed (var. Imperial). Certain rows, showing uniform stands and distributed over the seed-bed, were dusted on 5 January, 31 January and 25 February (the date of lifting) with Folosan or D.B.; others remained undusted. The beneficial effect of the two earlier dustings was obvious at the time of lifting, the control rows now showing many more gaps. The record of *living* plants at the time of lifting is given in summary in Table 5.

TABLE 5

Treatment	Large seedlings with no obvious lesions	Large seedlings with lesions	Small seedlings	Total
Control (av. of 7 rows)	37 (range 28–66)	22 (17–26)	15 (8–27)	74 (53–119)
Folosan (av. of 3 rows)	165 (139–215)	20 (18–21)	95 (54–168)	280 (222–328)
D.B. (av. of 4 rows)	140 (124–156)	33 (12–57)	86 (53–123)	259 (238–284)

Two applications of the dust were not effective in eliminating disease, but the beneficial effect appears in the first column of the table which shows that there were

many more 'plantable' seedlings in the treated rows. The second column does not favour the treatments, but these figures are unimportant; the essential point is that many of the plants in the control rows were already dead, as shown in the column of totals. The large number of small plants in the treated rows is to be set down to overcrowding, as the rate of seeding was high (1 g. per row of 4 yd.).

A sixfold replicated planting was made of the large seedlings which were apparently healthy, and this gave the following data of survival: Control, 37·5%; Folosan, 72·9%; D.B. 46·5%. Thus while the dusts were about equally effective in preserving the stand of seedlings in the seed-bed, Folosan gave a much higher survival of plants at maturity. It may have been that the final application of the D.B. dust was faulty. This can only be decided by further experiment.

Overwintering of plants in seed-beds

Throughout the experiments described above, six seed-beds were used year after year. The earlier sowings (*c.* 10 September) in these were removed during November, but the bulk of the later ones (*c.* 20 September) allowed to stand in the seed-bed until early spring (February–March). Additional seed-beds, which were laid down especially in seasons 1944–5 and 1945–6, were sited so as to obtain a range of soil conditions. These beds were drilled at the usual rate (0·25 g. seed to the yard run), with the varieties Imperial and Trocadero Improved, in rows about 9 in. apart, and as the object was to study overwintering in the seed-bed sowings were made in the latter half of September. To ensure even germination the beds were, when necessary, well watered on the day before sowing. Altogether forty-six such seed-beds have been studied in the course of the work.

(1) *Effect of seed treatment on emergence*

In September 1943, September 1944 and February 1945 a comparison was made between seed dusted with red cuprous oxide ('Cuprocide' at the rate of 1% of seed weight) and undusted seed. Twelve seed-beds were laid down for this test, involving altogether 101 row-by-row comparisons of seed treatment *v.* no seed treatment. Ninety-three of these gave an increased stand in the treated rows. In all of the twelve seed-beds, the average emergence per row was increased by the treatment, the increase ranging from 11·6 to 83·5% (av. 35%).

In a tenfold replicate experiment the effect of the standard dose (1%) of cuprous oxide was compared with that of an overdose (5%), with the following result:

Increase of stand on treatment with 1% cuprocide—15·2%,

Increase of stand on treatment with 5% cuprocide— 1·5%,

indicating clearly that 5% is above the optimum dosage.

These results confirm the statements of Ogilvie & Hickman (1938) in England and of several workers elsewhere, especially in America.

(2) *Progress of disease in seed-beds*

The first pathological effect which may appear is damping-off caused by *Rhizoctonia Solani*. This usually occurs within a few weeks of seedling emergence, in patches spreading along the rows. At this stage the young plants are carrying green cotyledons and are not subject to *Botrytis* attack. Entry of the damping-off fungus is normally through the hypocotyl and isolations readily give cultures of *Rhizoctonia Solani*. As time goes on *Botrytis* is found sporulating freely on the dead plants, but at this stage it is to be considered merely as a saprophyte.

Rhizoctonia damping-off has varied in intensity from one seed-bed to another to a marked degree. In many it has been negligible or absent; in some it has caused obvious gaps, and in one seed-bed (A) it has persistently destroyed the stand. In all the years of the experiments severe damping-off had begun in seed-bed A by the end of November at latest, and all the seedlings had disappeared by February. Addition of the standard fertilizers (K, P, N) had no effect, and the only treatments which gave an improvement were dusting the seed with cuprous oxide and addition of Folosan dust to the soil covering the seed. These improvements were merely temporary, the treated rows succumbing in the long run.

So far as could be seen the cropping history of seed-bed A was not materially different from that of other beds in which the disease was much less prominent. It remains for further research to determine what conditions lead to the establishment of *Rhizoctonia* in some pieces of ground and not in others.

Patches of yellowish slow-growing plants occur sporadically in the seed-beds throughout the winter. In these plants the tap root is rotted at the tip so that it appears as a brownish red stump, and the finer side roots are more or less browned and rotted. Isolations from such roots have regularly yielded a species of *Pythium*. Plants attacked in this manner are not killed immediately, if at all. As the winter advances the root system becomes more and more reduced, and the plant may finally lie on the surface of the ground where it dies; or when good growing conditions return in the spring it may strike root afresh from above the infected area and again establish itself. Nevertheless, growth is much retarded and such recovered plants are of little value for cropping purposes. The variety Trocadero is much more prone to this root trouble than is Imperial, a fact which largely accounts for its inferiority as a winter lettuce.

Most seed-beds are healthy in November, apart from an occasional patch of damping-off. Samples set out in the field at that time overwinter according to the nature of the ground into which they are planted (as shown above) and without any relation to the further history of the seed-beds from which the samples were taken. The deterioration of seed-beds, which takes place in greater or less degree as time goes on, is not under way by November.

The process of deterioration has been followed by making careful plans of seed-beds in November, noting where any gaps or damped-off patches occur. By

February the original gaps or patches have usually enlarged and new ones, sometimes many new ones, have arisen. By this time the stems of the plants are sheathed with the more or less withered and ragged leaf bases of withered cotyledons and first leaves. The predominant fungus at this stage is *Botrytis*, sporulating freely on all dead or moribund plants.

The rate of overwinter deterioration varies between seed-beds but is fairly constant for any one bed. Thus in contrast to the seed-bed A already mentioned, a seed-bed C has regularly carried through the winter a strong stand of good plants. Also there is a distinct indication that the best seed-beds for use in spring planting occur on heavier soil. It does not appear that the value of a particular seed-bed for overwintering seedlings is affected by such cultural details as whether or not the seed-bed had been freshly dug at the time of sowing, and whether its surface was loose or compacted. Addition of farmyard manure has also given no clear-cut result.

The condition of a seed-bed in early spring was assessed by lifting the plants row by row and, after rejecting small seedlings, sorting those of 'plantable' size into two groups: (*a*) with, and (*b*) without visible stem lesions. The advance of deterioration is seen by the increase in the percentage of plants with lesions, and later by the presence of fewer living plants. Two stages of the process have already been illustrated (Table 5) in another connexion. Plantings were made of seedlings without visible stem lesions and their survival determined. A correlation was found between the percentage of lesioned seedlings rejected, and the lower survival rate of the apparently sound ones after planting.

The data of seven such experiments with Imperial, the first three in the spring of 1943 and the others a year later, illustrate this correlation (Table 6). In each experiment the plants came from different seed-beds of the same age, and the plantings were simultaneous and replicated in the same plot.

Table 6

Exp.	% seedlings rejected	% survival of seedlings planted
I	19·5	74·4
	0·3	96·3
II	20	80
	16·7	91
	2·1	96·5
III	14·6	79·6
	1	98·6
IV	38	39·5
	4·2	72
V	6·6	74·3
	1·1	93·1
VI	27	63·2
	3·8	92·4
VII	27	76
	4·2	91

This result suggests that the process of sorting fails to reveal small or incipient lesions, hidden among the withered leaf bases, and that the further deterioration has gone, the higher the percentage of already affected plants among those apparently sound. It is with seedling plants in this condition that a single dusting with Folosan at the time of transplanting often gives a much increased survival.

(3) *Effect of ridging the seed-bed*

Studies in 1944–5 and 1945–6 showed that when the seed-bed is prepared in the form of a series of ridges, the progress of deterioration is less rapid with seedlings grown in the hollows than on tops of the ridges. Sowings on the flat, as in the usual manner, tend to behave intermediately.

A single experiment of this nature was set up in the winter of 1943–4, so that if there should be a cold spell with snow, the plants in the hollows would obtain some protection from frost and might overwinter better on that account. During that winter there was negligible snow, and when the seed-bed was dismantled in March there was no obvious difference between ridges and hollows in the numbers of plants present. Grading of the seedlings for incidence of disease, however, showed that the percentage of visibly diseased plants was regularly greater on the ridges than in the hollows, as the following figures show:

Hollows:	av. plants per row, 200;	% diseased, 6·3.
Ridges:	av. plants per row, 207·5;	% diseased, 24·9.

In the two following winters seventeen seed-beds were set up to test this point further. The distance between consecutive ridges was about 1 ft. and the depth from ridge to hollow 4–6 in. The lay-out of such a seed-bed was on the following plan, F, R and H indicating a row on the flat, on the ridge and in the hollow respectively, F, F, R, H, R, H, R, repeated 2–4 times according to the length of bed available. If one calls the above succession of rows a unit, there were twenty-five such units sown with Imperial, the drills being about 4 yd. long, and a somewhat smaller number sown with Trocadero. All the sowings were made in the second half of September and the condition of the seed-beds recorded in November–December and in February–March.

Deterioration of the seed-beds in the early spring proceeded more rapidly on the ridges than in the hollows, the plants on the flat being intermediate. Table 7 contains the summarized records for four seed-beds of var. Imperial. These are chosen to illustrate the range of effects observed.

In seed-bed I deterioration had not proceeded to the length that many plants were dead, but its oncoming was shown by the presence of plants with visible lesions. Seed-bed IV was in the most advanced stage, II and III being intermediate.

In twenty-four out of twenty-five such comparisons with var. Imperial, the hollows gave a larger number of living plants than the ridges, and the percentage

of diseased plants was always greater on the ridges than in the hollows (as illustrated in Table 7).

Table 7

Seed-bed	Av. no. living plants per drill	% diseased
I: H	199	14
R	170	17·6
II: H	155	16·4
R	76	26·3
III: H	97	13
R	45	33
IV: H	74	17·5
R	22	59

In parallel tests with var. Trocadero the same type of result was obtained, but usually not so clearly. With this variety there is the complication arising from its susceptibility to a root disease, so that the stands are usually much thinner and more irregular.

In the spring of 1946 there were two large seed-beds in which deterioration was in its early stages. The stands of plants in hollow, ridge and flat looked equal, and the proportion of dead plants was negligible. Half of each bed was of Imperial and half of Trocadero. The seedlings were lifted, recorded, and four plantings, two of Imperial and two of Trocadero, made in fourfold replication. The records of two typical batches are given in Table 8.

Table 8

Variety	Seedlings per drill	% diseased	% survival to maturity of apparently healthy seedlings
Imperial: H	168	4·1	72·3
F	180	7·9	70·7
R	148	18	51
Trocadero: H	164	5·5	78
F	156	6·6	75
R	135	8·5	67·2

The most noteworthy point brought out by Table 8 is the evidence that deterioration is already well established in the ridges. It will also be seen that the data of the penultimate and last columns again illustrate the result shown in Table 6.

Discussion

The value of the winter lettuce crop rests largely upon its earliness, maturing as it does some 2–4 weeks before the spring lettuce crop which has been overwintered in cold frames and planted out in early spring. Thus it follows the expensive and limited crop which has been matured under glass, and meets a very active demand, especially in years of warm early spring weather. The records of market prices

obtained during the run of these experiments were no doubt somewhat abnormal on account of wartime conditions, but a similar trend is likely to be seen in any year, viz. a fairly rapid fall in prices, usually in the first half of May, but varying somewhat according to seasonal conditions. Thus lettuces marketed at the beginning of May often reach 10*s*. to 14*s*. per box of twenty-four, and a fortnight later the price may have fallen to 4*s*. to 5*s*. Hence the premium upon early maturity.

The experimental results given in this paper show what measure of earliness is obtained by different methods of culture, and it has been clear throughout that the most valuable crop was that obtained from the November plantings, provided the final stand was reasonably good. It was strongly emphasized that a light sandy soil was unsuitable for this purpose. Though no comparisons were made between crops transplanted in November from seed-beds and those drilled in September in the field where they are to mature, it seems likely that the latter, not having suffered a check from transplanting, would mature earliest of all. One can, however, visualize certain drawbacks to the drilling method. A careful comparison between the two, especially with reference to stand of plants, earliness of maturity and incidence of damage from pests and fungi, would be of distinct horticultural interest.

The value of early spring plantings from seed-beds sown in the latter half of September is that a succession of lettuce crops is thereby maintained. It has been shown that considerable variation exists between different pieces of ground in their capacity to furnish good stands of seedlings in early spring. Survival to maturity of spring-planted seedlings can also be much enhanced by a single fungicidal treatment at the time of transplanting. The means therefore exist for improving this method of culture, but further research is required before precise recommendations can be formulated. An important point is that the spring planting should be at the earliest moment—in February if possible. Delay in transplanting means delay in maturing, together with the risk, in a typical winter variety such as Imperial, that a proportion of the plants will run to seed without hearting properly at all.

The succession is then taken up with crops which have overwintered in cold frames and are normally planted out in March, and finally with those which are drilled in the spring in the open field where they are to mature.

The chief problem of scientific interest emerging from this work relates to the effect of soil type upon overwintering of lettuce seedlings. By far the most important killing agent is the fungus *Botrytis cinerea* which typically enters the plant by way of moribund tissue. As the lettuce plant does not cut off its old leaves by an absciss layer, with the result that the stem of an overwintered plant is sheathed with ragged leaf-base remains, and as *Botrytis* is such a ubiquitous fungus, the wonder rather is that any plants survive at all over the typically humid winter months. The experience gained in a number of severe winters has emphasized that winter lettuce is very resistant to frost as such, but the possibility exists that a period of freezing may lower the resistance of the lettuce stem to *Botrytis* which could then gain entry

if the frost period was followed by weather suitable to the fungus. Preliminary experiments, in which the soil surface between rows in seed-beds has been covered with a straw mulch, have furnished some interesting pointers in this connexion. Overwintered seedlings from the mulched rows showed whiter roots and in general were stronger and healthier. This suggests that protection of the root system from frost is an aid to overwintering and it would be easy to interpret the differential behaviour of hollows and ridges described in the preceding section in terms of this view.

On the other hand, an explanation may be sought along the lines of micro-biological antagonism. The entry of *Botrytis* into the lettuce stem being by way of the decayed leaf bases, anything which encouraged the development of saprophytes in these dying parts would help in preventing *Botrytis* from reaching the stem. Soiling up of the leaf bases would tend to assist in this action, and this may be the explanation of the better survival of seedlings in hollows than on ridges. The effects of straw mulching which have been briefly referred to and the differential values of light and heavy soils for overwintering which have been described in an earlier section may possibly also be explained on the same hypothesis. Further work is being directed to the elucidation of this problem.

The writers wish to express their indebtedness to Miss M. J. Smieton, Dr I. F. Storey and Mr R. K. S. Wood for assistance in these experiments, especially during the earlier years.

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(*Received* 11 *October* 1947)

STUDIES ON THE RELATIONSHIP BETWEEN EARTHWORMS AND SOIL FERTILITY

III. THE EFFECT OF SOIL TYPE ON THE STRUCTURE OF EARTHWORM POPULATIONS

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Observations on earthworm populations in the Carse of Stirling indicate that there are definite differences according to soil type both in total population numbers and in the relative numbers of each species present. The light and medium loams appear to carry higher total populations than the heavier clay types or the more open gravelly sand or alluvial types. Within and between the respective soil-type populations, species numbers are variable, but the actual numbers present on a soil type do not necessarily indicate the relative importance of each species. In all cases *Allolobophora caliginosa* is the dominant species, being more numerous on the light loam than elsewhere. *A. longa* is subdominant and forms a substantial percentage on the medium loam and clay. On the light loam *A. longa* is high in number, but forms a smaller percentage of the total population because of the abundance of *A. caliginosa*. *A. longa* is also of reduced importance on the open soils—gravelly sand and alluvium. This species does not differ very much from *Lumbricus rubellus* and *L. terrestris*, as a percentage of the population on these two later soils and the light loam. The percentages of these two species are similar within populations, though much lower on the clay than on the other soils, and the total *Lumbricus* population number is highest on the light loam and lowest on the clay. There is a fair similarity between the two populations on the two open-textured soils, in both numbers and percentages.

Areas of acid, natural pastures contain earthworm populations low in number and species variety. Some of the species typical of these areas are small active species, e.g. *Dendrobaena rubida, D. octaedra* and *Bimastus eiseni*, which are not found in well-established field populations. When agricultural treatment improves an area the acid-pasture species eventually decrease in relative number and importance as the species normally associated with good pasture land become established and build up their populations.

Introduction

This paper deals with investigations in the Stirling district of Scotland in autumn 1945 and 1946, on the variation of the earthworm fauna with soil type, and on the effect of agricultural improvement on the fauna of natural pasture.

The only previous reference to the distribution of the Lumbricidae in central Scotland is by W. Evans (1910), giving a list of species in the Forth area. Unfortunately, he identified his specimens according to Friend, and as many of Friend's descriptions have since been proved to be inaccurate, Evans's list is of doubtful validity. Nevertheless, his work does show that ten to fifteen species were present in this area.

In England, Evans & Guild (1947) have determined the structure of the earthworm populations of different Rothamsted fields. On the pasture fields of this farm

nine species of earthworms were identified, their number and proportions varying according to the history of the pasture, e.g. permanent, semi-permanent or recent. Table 1 shows the results obtained on three Rothamsted fields during the survey of March 1946 (Evans & Guild, 1947).

TABLE 1. *Adult and total populations on three pasture fields at Rothamsted, on a percentage basis*

	New Zealand (1 yr. grass)		Pastures (7 yr. grass)		Parklands (Permanent grass)	
Species	Adult	Total	Adult	Total	Adult	Total
Allolobophora nocturna	18·4	25·5	5·0	7·4	27·4	36·2
A. caliginosa	27·3	25·5	28·6	17·5	50·6	25·6
A. chlorotica	42·4	34·8	50·4	13·1	9·5	3·5
Eisenia rosea	6·1	10·0	15·1	6·6	6·6	3·5
Lumbricus terrestris	6·1	4·3	0·8	55·2	1·2	29·8
L. rubellus	0	0	0	0	0·6	1·0
L. castaneus	0	0	0	0	3·0	0·3
Octolasium cyaneum	0	0	0	0·2	1·2	0·3

The Rothamsted farm soil is a dark brown, silty medium loam, and so no comparisons between populations on different soil types, or between natural and agriculturally developed land, were possible.

SOILS OF THE DISTRICT

In central Scotland a number of distinct soil types occur within a comparatively small area, as shown by the soil map prepared by the Macaulay Soil Institute, Aberdeen. For the purpose of these investigations only the broader classification of texture was employed.

The soil types sampled were as follows:

(*a*) Silt-clay (Carse of Stirling).
(*b*) Medium loam (Campsie Fells).
(*c*) Light loam (Ochil Hills).
(*d*) Gravelly sand (Carse-Ochil Foothills).
(*e*) Alluvium (Carse of Stirling).

Survey 1945

The technique of surveying used was that which had been developed and proved successful at Rothamsted (Evans & Guild, 1947).

A preliminary survey was carried out in October 1945, at the estimated height of the autumn earthworm activity, i.e. when the soil temperature was about 50° F. and the soil-moisture percentage was not a limiting factor. A total of 4100 worms from 140 samples taken on four different soil types—clay, alluvium, medium and light loams—indicated that there were differences between the earthworm faunas of these different soils, both in total numbers and in the proportion of different species within the populations.

Survey 1946

High-quality permanent pasture

The main survey was made in October 1946, again at the estimated peak of autumn earthworm activity, and the soil types were sampled as shown in Table 2.

TABLE 2. *Sampling data, October* 1946

Soil type	Samples	Approximate acreage sampled
Silt-clay	62	40
Medium loam	44	40
Light loam	42	40
Gravelly sand	50	40
Alluvium	60	35
	258	195

These different soils all occurred within an area approximately 3–4 miles by 2 miles, so that differences between the populations to be discussed are almost certainly due to differences in soil type, and not to variation in distribution over large land areas.

TABLE 3. *Effect of soil type on earthworm populations (numbers in thousands per acre)*

Species		Clay	Medium loam	Light loam	Gravelly sand	Alluvium
A. longa	*Adult*	*14·4*	*11·5*	*9·0*	*11·1*	*5·9*
	Total	49·8	68·8	65·8	26·3	31·6
A. caliginosa	*Adult*	*34·4*	*23·6*	*34·6*	*9·0*	*17·7*
	Total	70·3	81·7	146·1	36·5	59·4
A. chlorotica	*Adult*	*3·5*	*0·1*	*0·6*	*2·7*	*3·6*
	Total	6·0	0·1	0·9	5·2	8·0
A. icterica	*Adult*	*1·5*	—	—	—	—
	Total	4·5	—	—	—	—
E. rosea	*Adult*	*7·0*	*1·8*	*8·1*	*3·4*	*4·9*
	Total	9·7	4·5	12·2	5·6	6·2
L. castaneus	*Adult*	*0·2*	*6·4*	*5·3*	*5·7*	*0·2*
	Total	1·3	10·4	12·4	8·6	3·4
L. rubellus	*Adult*	*2·5*	*5·8*	*20·4*	*8·8*	*7·2*
	Total	7·6	22·5	64·4	27·7	29·2
L. terrestris	*Adult*	*4·4*	*5·1*	*4·9*	*4·4*	*6·6*
	Total	12·5	34·3	47·1	30·4	36·9
L. festivus	*Adult*	—	—	—	—	*1·8*
	Total	—	—	—	—	2·3
O. cyaneum	*Adult*	*0*	*0·7*	*0·4*	*0·6*	*1·1*
	Total	1·0	3·1	4·5	2·2	2·6
O. lacteum	*Adult*	*0·2*	—	—	—	*0·2*
	Total	0·2	—	—	—	0·2
D. mammalis	*Adult*	—	—	—	—	—
	Total	—	—	—	—	—
D. octaedra	*Adult*	—	*0*	—	*0*	—
	Total	—	0·1	—	1·6	—
D. rubida	*Adult*	—	*0·3*	*0·9*	*2·0*	—
	Total	—	0·6	2·8	2·7	—
	Total	163·8	226·1	356·0	146·8	179·8

Table 3 shows the numbers of worms of each species, adults and totals, obtained on the different soil types. Standard errors could not be attached to these figures because, although it was possible to keep the total samples from each field surveyed separate, transport difficulties made it impossible to keep individual samples separate. So the significant differences between populations were estimated by the method of χ^2 (Snedecor, 1942).

These populations are comparative, and are equivalent to the active populations, as shown by Evans & Guild (1947), and not the total populations.

Table 4 gives the values of χ^2 for the different populations, and the levels of significance.

TABLE 4. *Values of* χ^2

Soil type	χ^2	Significance
Light loam (356)* and medium loam (226)*	29·0	Highly significant
Light loam (356) and alluvium (180)	57·0	Highly significant
Light loam (356) and clay (164)	69·0	Highly significant
Light loam (356) and gravelly sand (147)	87·0	Highly significant
Medium loam (226) and alluvium (180)	5·6	Significant
Medium loam (226) and clay (164)	9·8	Highly significant
Medium loam (226) and gravelly sand (147)	16·7	Highly significant
Alluvium (180) and clay (164)	0·8	Not significant
Alluvium (180) and gravelly sand (147)	3·3	Not significant
Clay (164) and gravelly sand (147)	0·9	Not significant

χ^2 for 1 degree of freedom at 5 % level = 3·84.
χ^2 for 1 degree of freedom at 1 % level = 6·64.

* Figures in brackets refer to the population in 1000's per acre.

This table shows that the population on the light loam (356) is significantly higher than those on the other four soil types, and that the population on the medium loam (226) is significantly higher than those on the remaining three types. The populations on the alluvium (180), gravelly sand (147) and the clay (164) are not significantly different.

In addition to the differences between the total population numbers, Table 3 shows that there are differences in species numbers between populations.

As the population on the light loam is so much higher than on each of the other four soils, it might be expected that the number of each species present would also be significantly higher, but in fact this is true for two species only—*Allolobophora caliginosa* (146) and *Lumbricus rubellus* (64).

The population ratios for *Allolobophora caliginosa* are:

A. Gravelly sand (37)	1·0
B. Alluvium (59)	1·6
C. Clay (70)	1·9
D. Medium loam (82)	2·2
E. Light loam (146)	4·0

Significant differences E > D = C = B > A.

No other species exceeds *A. caliginosa* in number on any of the soils, and this is also the case with the population on certain permanent pasture fields at Rothamsted.

The deep-burrowing species *A. nocturna* which is common at Rothamsted was not encountered in either of the Scottish surveys, but its counterpart in the population is *A. longa*. These two species are similar in size, appearance and habit and probably have similar functions in the soil. Like *A. caliginosa*, *A. longa* appears to be most numerous on the loams, but the population on clay (50) is not significantly different ($\chi^2 = 3{\cdot}0$). These three *A. longa* populations are significantly higher than those on the gravelly sand (26) and the alluvium (32), possibly because these free-draining soils are unsuitable for this species.

With *A. chlorotica* the position is rather the reverse, as the populations on the two loams—0·1 on medium loam and 0·9 on light loam—are very much lower than those on the gravelly sand (5·2), alluvium (8·0) and clay (6·0). As at Rothamsted, *Eisenia rosea* is not a numerically important species in field populations. The numbers given in Table 3 indicate that the distribution of this species is not greatly affected by soil type.

The total *Lumbricus* species population is significantly higher on the light loam (124) than on the other four types, while the *Lumbricus* species population on the clay (21) is significantly lower than on the other types, χ^2 for clay and gravelly sand = 24. All three species—*L. rubellus* (64), *L. castaneus* (12) and *L. terrestris* (47)—are most numerous on the light loam, but only for *L. rubellus* are the numbers significantly higher than on any of the other types, being twice that of the next highest population—alluvium (29). The *L. rubellus* number on the clay (8) is significantly lower than on the four other types. Again with *L. terrestris* the number is significantly lowest on the clay (13). The numbers of *L. castaneus* are low on all soil types, that on the clay (1·3) being significantly lower than the populations on the medium loam (10·4), gravelly sand (8·6) and light loam (12·4), but not significantly different from that on alluvium (3·4).

L. festivus was found only on the alluvial soil (2·3) and is either absent or scarce on other soils. Why this should be so is not at all clear, because the species has been recorded from different soils in other parts of the country. It may be that the conditions of this particular soil were suitable, and that as a result there has been local establishment of the population without spread to other soils. The alluvium on both sides of the River Allan contains this species, but it was not encountered at all on immediately adjacent soils of a different type.

Of the remaining Rothamsted pasture species only *Octolasium cyaneum* occurs on all the Scottish soils sampled, and in no instance are its numbers high—4·5 on light loam being the maximum. *O. cyaneum* occurs only on clay (0·2) and on alluvium (0·2). *Dendrobaena octaedra* and *D. rubida* occur infrequently on the medium and light loams and on the gravelly sand and do not appear to form an important part of the population on established pasture land. The succeeding section on Ballochraggan surveys will show that the occurrence and distribution of these species is closely associated with soil conditions and agricultural practice.

Table 5 gives the species numbers as a percentage of the total population for each soil type.

TABLE 5. *Percentages of species on different soil types*

Species	Clay	Medium loam	Light loam	Gravelly sand	Alluvium
A. longa	30·4	30·4	18·5	17·9	17·6
A. caliginosa	42·9	36·1	41·0	24·9	33·0
A. chlorotica	3·7	0·1	0·3	3·5	4·5
A. icterica	3·3	0	0	0	0
E. rosea	5·9	2·0	3·4	3·8	3·5
L. castaneus	0·9	4·6	3·5	5·9	1·9
L. rubellus	4·6	10·0	18·1	18·9	16·2
L. festivus	0	0	0	0	1·3
O. cyaneum	0·6	1·4	1·3	1·5	1·4
O. lacteum	0·1	0	0	0	0·1
D. octaedra	0	0·1	0	1·1	0
D. rubida	0	0·3	0·8	1·8	0

On the light loam it is seen that *Allolobophora caliginosa* is significantly higher in numbers than on any other area, but Table 5 shows that as a percentage of the light loam population (41%) it is not significantly different from the corresponding percentages on clay (43%), medium loam (36%) and alluvium (33%). On the gravelly sand the species forms only 25% of the population. In all five populations, however, *A. caliginosa* forms the greatest percentage.

In two soil types, a second species forms nearly as high a percentage of the population as *A. caliginosa*. These are the gravelly sand where *Lumbricus terrestris* forms 20·7%, and the medium loam where *Allolobophora longa* forms 30·4%, compared with 24 and 36% for *A. caliginosa* respectively. *A. longa* forms 30·4% of the population on the clay, as well as on the medium loam, but in this case *A. caliginosa* is significantly higher (43%). On the light loam also, *A. longa* is subdominant (18·5%), but much lower than *A. caliginosa* (41%). The open-textured soils have reduced percentages of *A. longa*, 18% on both the gravelly sand and the alluvium, and in these cases the subdominant species is *Lumbricus terrestris* 21 and 25% respectively.

L. rubellus and *L. terrestris* form similar percentages within each population, and in no case is there a difference of more than 5·2% between the two species (non-significant). Inclusively, the *Lumbricus* population on clay (13·1) is very much lower than on the remaining soils. As with *Allolobophora caliginosa*, the highest *Lumbricus* population number occurred on the light loam, but as a percentage, 34·8, it is not significantly different from that of the medium loam, 29·8, and alluvium, 39·9. The *Lumbricus* population percentage on the gravelly sand, 45·5, is not significantly higher than that of the alluvium, but is significantly higher than that of the other three types.

Of the remaining species, none formed more than 6% of the population on any of the soil types sampled, and cannot be considered as important. With some

exceptions, the species *Allolobophora chlorotica*, *Eisenia rosea*, *Lumbricus castaneus*, and *Octolasium cyaneum* form similar percentages between populations.

Since these species are present in comparatively low numbers it is difficult to tell whether soil type influences their distribution to any extent, but even so, *Allolobophora chlorotica* is very low on the two loams and *Lumbricus castaneus* and *Octolasium cyaneum* are fairly low on the clay.

Acid natural pastures

Ballochraggan Hill Farm. These investigations were carried out at Ballochraggan Hill Research Farm, Lake of Menteith, Perthshire. Four different areas, A, B, C and D, were sampled, as described below, to see how the population varied between natural, untreated hill pasture, and pasture which had been subject to treatment and cultivation.

A. Untreated hill pasture rising from 150 to 600 ft. The soil was acid, and of varying depth. Bracken was present, though much reduced by cutting, and the soil surface bore a thick mat of vegetation. Very little earthworm casting was present.

B. This area (Pine Tree Field and upper Swamp Field) was recently brought under pasture cultivation, Pine Tree Field having been ploughed in July 1944 and Swamp Field in June 1945. Both had been treated with lime and had received various dressings of fertilizers.

C. This area (Cottage Field and Arntamie Field) was ploughed in November 1942. It was well cultivated by ploughing, disking, harrowing and rolling, and received various dressings of lime and fertilizers between 1943 and the time of sampling.

D. An area at the base of the hill, which was well limed in 1942, and had received fertilizer treatment in 1944. Cattle tended to congregate in this area, which was therefore also fertilized by their droppings.

On B and C the herbage was much improved compared with A, and included much clover as well as the common pasture grasses. The herbage on D was also much better than that on A, and included much clover. The soil of D was much wetter than on A and there was less surface mat. Casting on D was considerable in parts, but it was of finer material than that commonly associated with normal casting produced by the *Allolobophora longa-A. nocturna* group.

The results of these surveys are given in Table 6. First it will be seen that the population on A is very much lower than those of the other three areas, indicating that acid soil supporting rough herbage is not a suitable environment for a large, well-balanced population. On B and C, where the soil had been subject to cultivations and treatment for a few years, the population has increased about four times. There is no significant difference between the total populations of B and C.

Area D, which resembles a good pasture, has a population over eight times that of area A, showing the beneficial effect of improvements in environmental conditions. If the numbers of *Dendrobaena rubida* are subtracted from the total

population of area D (as this species is not typical of well-established pastures), the remaining population, (363),* is little different from the population of the similar light loam soil, (356), of the soil-type survey. This point will be referred to again later.

TABLE 6. *Ballochraggan—populations in thousands/acre*

		A		B		C		D	
			%		%		%		%
A. longa	*Adult*	—		—		—		—	
	Total	—		—		—		—	
A. caliginosa	*Adult*	—		*1·9*		*8·1*		*31·1*	
	Total	—		5·9	2·7	22·8	12·2	99·6	22·5
A. chlorotica	*Adult*	—		—		*0·5*		*31·1*	
	Total	—		—		0·8	0·4	47·7	10·8
E. rosea	*Adult*	—		*0·3*		*0·8*		*6·2*	
	Total	—		0·3	0·1	1·6	0·9	7·3	1·6
L. terrestris	*Adult*	*0*		*1·2*		*0·8*		*1·0*	
	Total	0·5	1·0	3·1	1·4	5·7	3·0	18·7	4·2
L. rubellus	*Adult*	*11·4*		*37·3*		*24·6*		*76·7*	
	Total	18·2	35·3	53·8	24·5	65·0	34·7	155·5	35·1
L. castaneus	*Adult*	*1·0*		*15·9*		*4·4*		*8·3*	
	Total	2·6	5·0	24·3	11·1	13·7	7·3	27·0	6·1
A. rubida	*Adult*	*24·4*		*77·1*		*42·6*		*64·3*	
	Total	28·6	55·5	129·7	59·2	76·4	40·7	79·9	18·0
D. octaedra	*Adult*	*1·6*		*1·2*		*0·3*		*1·0*	
	Total	1·6	3·1	1·2	0·6	0·5	0·3	1·0	0·2
O. lacteum	*Adult*	—		*0·3*		*0*		*5·2*	
	Total	—		0·9	0·4	1·0	0·5	6·2	1·4
Total		51·5		219·2		187·5		442·9	
Total adults only		*38·2*		*135·2*		*82·1*		*224·9*	

The deep-burrowing species, *Allolobophora longa*, is not encountered at Ballochraggan. Results at Rothamsted suggest that *A. longa* is a slowly reproducing species which takes a long time to establish itself in a population. Therefore it is probable that the improved areas of Ballochraggan (B, C, D—4 years at maximum) have not been under treatment long enough for the species to become established.

The changes in the species complex are well shown in Table 6. The typical pasture species, *A. caliginosa*, is absent from the hill area A, but it has become established under improved conditions, being 5·9 on B, 22·8 on C and 99·6 on D. In the last-named area *A. caliginosa* is subdominant to *Lumbricus rubellus*, (155·5), whereas it was shown to be dominant on the established pastures of the soil-type survey, but the figures show that the proportionate increase of *Allolobophora caliginosa* is more rapid than that of *Lumbricus rubellus*—5·9, 22·8, 99·6 against 53·8, 65·0, 155·5—and it is probable that it will become dominant in the future. On A, *Lumbricus rubellus* (18·2) forms 35·3% of the population, and although the percentage falls to 24·5 on area B as a result of a rapid increase of *Dendrobaena rubida* numbers, *Lumbricus rubellus* has increased to 53·8 on this area. There is

*=population numbers in thousands per acre.

a further increase on C up to 34·7% of the population (65·0), while the species has become dominant on D, forming 35·1% of the population (155·5). The comparative changes in proportions of the population for *Allolobophora caliginosa* and *Lumbricus rubellus* are:

A. caliginosa (%)	0	2·7	12·2	22·5
L. rubellus (%)	35·3	24·5	34·7	35·1

The remaining *Lumbricus* species, *L. castaneus* (2·6) and *L. terrestris* (0·5), are present, but in very low numbers on area A, and both have increased substantially on areas B, 24·3 and 3·1 respectively, and C, 13·7 and 5·7 respectively. The initial increase of *L. castaneus* on area B has not been maintained, and the numbers have fallen to 13·7 on C, but under the conditions of D this species has again increased (27·0). The *L. terrestris* numbers do not show this drop, but have increased steadily from 0·5 on A up to 18·7 on D. Thus on A, which is also the light-loam type of soil, the total *Lumbricus* population number is only 21·3. This is about one-sixth of the equivalent population on the light loam in the soil-type survey, and is in fact little different from the lowest *Lumbricus* pasture population—21·4 on the clay. But on D the great increase in the *Lumbricus* number to 201·2 shows the very favourable conditions of this area for these species.

The species *Allolobophora chlorotica*, *Eisenia rosea* and *Octolasium lacteum* each show a similar trend to *Allolobophora caliginosa* and *Lumbricus rubellus*. No individuals of these three species were found on A, but *Eisenia rosea* and *Octolasium lacteum* have become established on B at 0·3 and 0·9 respectively, and have further increased on C to 1·6 and 1·0, and on area D to 7·3 and 6·2 respectively. *Allolobophora chlorotica* has not become established on B, but it has made its appearance on C—0·8. Previously it has been stated that this species is not normally prevalent on soils carrying permanent pasture, and it is interesting to note the tremendous increase in numbers of this species on D—47·7—where it forms 10·8% of the population. This is twice the percentage which *A. chlorotica* formed of the alluvium population in the soil-type survey, where it was most numerous.

The small, active species, *Dendrobaena rubida*, is very important when considering the populations of acid soils. Together with *D. octaedra* and *Bimastus eiseni*, this species seems typical of acid conditions, where the populations are low in number and variety of species. Under improved soil conditions, when larger populations are built up, *Dendrobaena rubida* decreases in importance and is succeeded by other species. The Ballochraggan samplings show this well. On area A, *D. rubida* was the dominant species (28·6), forming 55·5% of the population. On B, the numbers of *D. rubida* increased enormously (129·7), and the percentage to 59·2. This was to be expected because the improved conditions of B would be most suitable for the rapid multiplication of an active species like *D. rubida*, which was able to build up a high population quickly, much more rapidly than the other pasture species which are either slower reproducers, or have had to establish and build up a population from near zero. At this stage *Lumbricus rubellus* has increased from 18·2 to 53·8 and

Allolobophora caliginosa, previously absent, has established a small population (5·9). However, on C, if *Dendrobaena rubida* is omitted, the remaining population has increased from 89·5 to 111·1 (not quite significant by χ^2), and this increase is due to an increase in the pasture species. On the other hand, *D. rubida* has not increased similarly, but has decreased to 76·4 (40·7% of the population). The decrease in the importance of *D. rubida* is even more marked on D, for here the pasture species have considerably increased their number, while *D. rubida* has only maintained its number at 79·9 and has dropped to 18·0% of the population—less than either *Allolobophora caliginosa* (22·5%) or *Lumbricus rubellus* (35·1%).

Pendreich Reservoir. A similar survey was carried out at Pendreich Reservoir in Stirlingshire, and four areas were sampled as follows:

E. Acid, peaty soil, without agricultural treatment, and bearing a cover of coarse herbage and heather.

F. Similar to area E, but with very shallow soil, 1–6 in. deep, and very dry.

G. An area, once enclosed as pasture, but now 'gone-back'. The soil was fairly acid, but the herbage contained no heather, and although the grass was fairly long, it contained some clover and was of better quality than that of areas E or F.

H. An enclosed field area, with good quality herbage, containing much clover. The soil was not acid.

On these areas the position is the same as at Ballochraggan, as is shown by the results given in Table 7.

TABLE 7. *Pendreich Reservoir—population in thousands/acre*

		E		F		G		H	
			%		%		%		%
A. longa	*Adult*	—		—		—		*0*	
	Total	—		—		—		4·7	2·0
A. caliginosa	*Adult*	—		—		*7·3*		*26·0*	
	Total	—		—		22·3	22·3	78·9	34·0
A. chlorotica	*Adult*	—		—		—		*1·0*	
	Total	—		—		—		1·0	0·4
E. rosea	*Adult*	—		—		*1·0*		*7·8*	
	Total	—		—		2·0	2·0	17·7	7·6
L. terrestris	*Adult*	*0*		—		*0*		*1·6*	
	Total	0·3	0·6	—		10·4	10·4	23·4	10·1
L. rubellus	*Adult*	*3·6*		*0·5*		*7·3*		*4·7*	
	Total	21·6	42·4	4·2	17·1	26·0	26·0	61·8	26·6
L. castaneus	*Adult*	*0*		*0*		*1·6*		*7·3*	
	Total	0·5	1·0	2·1	8·5	7·3	7·3	33·2	14·3
D. rubida	*Adult*	*3·9*		*4·2*		*14·0*		*3·1*	
	Total	8·3	16·6	6·8	27·6	26·0	26·0	5·2	2·2
D. octaedra	*Adult*	*3·1*		*1·6*		*0·5*		*0*	
	Total	7·3	14·6	1·6	6·5	1·0	1·0	0·5	0·2
O. cyaneum	*Adult*	—		—		*0·5*		*1·6*	
	Total	—		—		5·2	5·2	5·7	2·5
B. eiseni	*Adult*	*6·0*		*5·7*		—		—	
	Total	12·4	24·8	9·9	40·2	—		—	
Total		50·0		24·6		100·2		232·1	
Total adults only		*13·6*		*12·0*		*32·2*		*53·1*	

The untended acid areas have very low populations, that of E being similar to that of A at Ballochraggan, although another acid-soil species, *Bimastus eiseni*, is present at Pendreich. The population of F, 24·6, is even lower than that of E, as the population has to contend with the additional factors of thin soil and dry conditions. Immediately adjacent to F is the improved pasture area H, and there the treatment received has led to a considerable increase in the population (232·1). Area G was presumably at one time similar to H, but here the effect of neglect, with consequent deterioration in the herbage and the soil, has been to halve the population (100·2) compared with that of A. Nevertheless, the population of G is still equal to twice that of E and four times that of F.

The general trends of the species are the same as that of the Ballochraggan populations. The typical pasture species *Allolobophora caliginosa*, *A. longa*, *Eisenia rosea*, *Octolasium cyaneum* and possibly *Allolobophora chlorotica* (low numbers) are absent from E and F, but are all present on H. The presence of *A. longa* indicates that H has been an established pasture for longer than any of the Ballochraggan areas sampled. Also *A. chlorotica* was only present in low numbers (1·0), as was observed with the equivalent light-loam permanent pasture (0·9) in the soil-type survey.

The three *Lumbricus* species have all increased on H compared with their numbers on E and F, but *L. rubellus* (61·8) is subdominant to *Allolobophora caliginosa* (78·9) indicating again that the population on this area is better established than that on Ballochraggan D. The low number of *Dendrobaena rubida* (5·2) also indicates this. All species, except *D. rubida*, are reduced in number on G, particularly the pasture species. *D. rubida*, which was present on E (8·3) as 16·6% of the population and on F (6·8) as 27·6% of the population, has a population of 26 (26% of the population) on G where pasture species offer less competition than on H (*D. rubida* 5·2). This reduction of *D. rubida* in the established population of H indicates its probable trend on Ballochraggan D in the future. If this happens and *D. rubida* is reduced to inconsiderable numbers, then the population on D at Ballochraggan will not be significantly different from the light loam population in the soil-type survey (light loam 356·0: Ballochraggan D (minus *D. rubida*) 363·0). The remaining acid-soil species, *D. octaedra* and *Bimastus eiseni*, were both present on E and F, but follow the same trend as *Dendrobaena rubida* and are in fact absent from D.

I am indebted to the following farmers for permission to sample on their land, and for the interest which they have shown in the results of the work: Mr R. Graham, Airthrey Kerse, Bridge of Allan; Mr McNicol, Pendreich, Bridge of Allan; Mr T. Reid, Netherton, Bridge of Allan; Mrs McLaren, Touch Mollar, Cambusbarron; Mr J. Stirling, Craigton, Causewayhead. I am also indebted to Prof. K. W. Braid of the West of Scotland College of Agriculture for his invaluable help and encouragement in preparing the surveys at Ballochraggan.

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(*Received* 23 *August* 1947)

WIREWORM POPULATIONS IN RELATION TO CROP PRODUCTION

V. COMPARISONS BETWEEN FAILING AND SUCCESSFUL PLOTS

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(With 1 Text-figure)

It is well known that infestation is only one of a number of factors which determine the amount of wireworm damage occurring in a field. As an attempt to analyse the conditions under which crops fail from wireworms, thirteen fields showing both damaged and undamaged areas were studied. Data were collected on wireworm populations, chemical and mechanical composition of the soil, feeding reactions of the wireworms collected, agricultural history and treatment of the different plots and fields. In six only of the fields did the damaged areas coincide with areas of higher infestation. In the remainder, the failures occurred at low populations, and no marked differences in population could be detected between the failing and successful plots. The levels of population at which the failures occurred varied between wide limits in the different fields. Two of the edaphic factors investigated were associated consistently with the failing and successful plots; $CaCO_3$ was consistently higher and sand content consistently lower on the failing plots. Differences in these factors were particularly noticeable in those fields where differences in infestation were not detected. There is a suggestion from four of the fields that wireworm attack commonly follows sainfoin. None of the other factors showed significant relationships. Although the results suggest that differences in one or more of the factors, infestation, $CaCO_3$ and sand, are associated with differences in wireworm damage within fields, the differences between the fields cannot be explained so simply. Evidently, other factors, relatively uniform within single fields but varying from field to field, also influence the amount of wireworm damage. Consideration of the results in relation to wireworm and plant populations brings out the need for greater attention to the role of wireworm activity and the environmental factors which influence it. The importance of reliable methods for predicting wireworm damage to any methods of control is stressed.

Introduction

The relation between wireworm infestation and the success or failure of a crop has been discussed in a number of recent publications. Evidence that populations could be estimated from collections of *Agriotes* larvae in soil samples and subsequent damage predicted with a useful degree of probability was presented by Finney (1941). Subsequently, the Report of the Advisory Entomologists' Conference on the Wireworm Survey (1944) presented more extensive data, incorporating the

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results of 4 years' work on 25,000 fields. These results showed a correlation between crop result and estimated population for all crops commonly attacked by wireworms. To take one example: at populations classified as 'low', 'medium', 'high' and 'very high' in 1941, failures of spring oats from all causes amounted to 21, 36, 45 and 64% respectively; in 1942 the corresponding figures were 17, 24, 38 and 58%. The data in the report show that population estimates based on the sampling and counting methods used in the Survey do provide considerable guidance on the probability of crop success. It is noteworthy, however, that the farmer still has something between a 1 in 2 and a 1 in 3 chance of a successful crop at the highest infestations, even with so susceptible a crop as oats.

In a large-scale survey it is quite permissible to disregard the minority of cases in which anomalous results, i.e. failures at low populations and successes at high populations, occur. But their numbers are by no means negligible, and any comprehensive account of wireworm damage to crops must take note of them. It has always been realized, of course, that the wireworm population in any given field is only one of a number of factors which together determine the amount of wireworm damage that will occur. All observers engaged in the Survey could report striking instances of good crops grown in the presence of populations that seemed excessive, and utter failures occurring on fields whose wireworm populations were very low indeed. In some cases the apparent anomaly could be attributed to the fact that about one estimate in eight was expected to be too high or too low simply on statistical grounds. In other cases there was reason to believe that the anomalous result was due to exceptionally favourable or unfavourable conditions for growth, either in the nature of the soil or location of the field, or in the practical ability of the farmer and the resources at his disposal. Yet, in our experience, there were many cases for which no obvious explanation could be suggested for the 'low failure' or 'high success'.

In our work, this question of the relation between crop result and infestation arose more sharply when we began to pass from the large-scale survey to the intensive study of populations in a few selected fields, using an improved method of counting larvae free from the defects of hand-sorting (Cockbill, Henderson, Ross & Stapley, 1945; Ross, Stapley & Cockbill, 1947). In these fields, the degree of infestation was known accurately as a result of repeated samplings and the precise conditions on each field had been closely studied. Nevertheless, even in these circumstances, a high proportion of anomalous results occurred which could not be attributed to any of the usual factors. We decided, therefore, to investigate the relation between crop result and infestation on individual fields and to try to detect factors influencing the severity of wireworm attack in such cases, which might account for some of the anomalies observed. Moreover, evidence on the natural conditions associated with absence of damage on highly infested fields or with excessive damage in lightly infested fields might open up entirely new possibilities of control.

The typically uneven pattern of wireworm damage on many fields, usually described as 'patchy', seemed to present an opportunity for investigating the problem. Where failing and successful areas occur within a single field, it seemed desirable to know whether these differences in crop result simply reflect different levels of infestation or whether they reflect differences in the feeding activity of the larvae. Investigation of any cases in the second category might suggest some of the factors which influence the severity of wireworm attack on crops.

Procedure

In spring 1945 fields were investigated on which failing and successful areas were showing up distinctly. Data were recorded on the following points in both areas: (1) wireworm population; (2) plant density; (3) soil analysis; (4) feeding reactions of wireworms collected; (5) rate of collection of wireworms on the field; (6) previous history of the field; (7) observations on drainage, growing conditions and any other factors which might affect the crop result. Data on soil consolidation, obtained by using the compactometer developed by Culpin (1936), are not included because the results proved to be so variable and because consolidation is itself affected by the failure of a crop. The looseness of the soil so often associated with wireworm failures may be an effect caused by the failure rather than a factor contributing to it. Differences in this factor would be significant only if they could be detected before and not after the failure had occurred. Tests for differences in soil moisture were not made, because differences of the order which Evans (1944) and Lees (1943) associated with differences in the behaviour of *Agriotes* larvae were not to be expected between different parts of a single field.

In fields on which visible differences in crop result were showing up, four plots, each 2 sq. chains in area, were marked off, two where the crop was failing and two where it appeared normal. From each plot, fifty cylindrical soil samples were collected, 4 in. in diameter and 12 in. deep, and used for estimating the wireworm population. Plant density was estimated by counting the living plants in twenty rectangles, each 2 sq. ft. in area, distributed over each plot. Six soil samples were taken from each plot for chemical and mechanical analysis. Living wireworms were then collected for tests of feeding reactions, and the whole field was thoroughly surveyed and all information available about its previous history was recorded. Fields were chosen for the investigation only if the failure of the crop could be attributed without question to wireworm attack, i.e. if larvae could be found feeding on the plants. In one case, mentioned later, it was not certain that wireworms were the sole or primary cause of the failure.

In the laboratory the wireworm samples were treated by the washing and flotation process described in our first paper (Cockbill *et al.* 1945). The soil samples were sent to the Advisory Chemist for mechanical analysis and determination of pH, $CaCO_3$, available potash, available phosphate, total nitrogen, organic carbon, loss on ignition, loss on solution and moisture. Where sufficient numbers of

wireworms could be collected (these were sometimes difficult to find in the field), they were sent to Dr Crombie at the Zoological Laboratory, Cambridge, who tested their feeding reactions.

Results

Wireworm populations in successful and failing plots

From 21 March to 26 June 1945, thirteen suitable fields were located which showed wireworm damage occurring in patches. Ten of the fields were growing cereal crops (five wheat, three oats, one barley and one maize), two were sugar beet and one was reseeded grass. The results of the wireworm population tests on the failing and successful areas in these fields are shown in Table 1. It will be advisable to discuss first the application of these results to the problem under discussion, the relation between infestation and crop result.

Table 1. *Wireworm populations in successful and failing plots in fields* 1–13 (*thousands per acre*)

Field no.	Crop and past history	Plot no.	Condition of plot (successful or failing)	Plants per 2 sq.ft.	Population thousands per acre all sizes	Population thousands per acre 5 mm. and over	Difference F.–S. all sizes	Difference F.–S. 5 mm. and over
1	Wheat after sainfoin,	1	S.	81·2	440	420	900	910
(21. iii. 45)	barley and winter	2	S.	75·9	680	660		
	oats	3	F.	18·9	1390	1380		
		4	F.	12·6	1530	1520		
2	Wheat after trefoil,	1	S.	38·3	1080	700	1495	1275
(13. iv. 45)	barley, kale and	2	S.	33·6	780	580		
	swedes; wheat after	3	F.	6·0	2150	1770		
	sainfoin, barley, kale	4	F.	7·0	2700	2060		
	and swedes							
3	Wheat after old grass	1	S.	Too	1800	1250	2285	1390
(18. iv. 45)		2	S.	advanced	1710	1240		
		3	F.	for counts	4110	2650		
		4	F.		3970	2620		
4	Wheat after flax, oats,	1	S.	37·0	150	140	260	180
(27. iv. 45)	beet and old grass	2	S.	24·5	190	150		
		3	F.	6·3	380	280		
		4	F.	5·7	480	370		
5	Wheat after old grass	1	S.	Too	320	290	40	40
(14. v. 45)		2	F.	advanced	360	330		
				for counts				
6	Winter oats after	1	S.	78·3	710	690	30	10
(27. iii. 45)	fallow, wheat and oats	2	F.	24·1	740	700		
7	Oats after sainfoin,	1	S.	55·0	830	530	800	1080
(4. v. 45)	barley	2	F.	7·3	1630	1610		
8	Oats after sainfoin,	1	S.	62·2	1230	980	600	530
(17. v. 45)	barley	2	F.	10·5	1830	1510		
9	Barley after beans;	1	S.	69·2	400	350	180	−10
(9. iv. 45)	barley after clover	2	S.	92·1	380	340		
		3	F.	65·2	790	470		
		4	F.	64·2	350	200		
10	Maize after wheat,	1	S.	142·0	470	310	180	270
(22. vi. 45)	fallow and lucerne	2	F.	59·0	650	580		
11	Mangolds after wheat,	1	S.	—	370	320	30	30
(17. vi. 45)	fallow and lucerne	2	F.	—	400	350		
12	Beet after wheat, red	1	S.	3965*	850	430	5	100
(24. v. 45)	clover and barley	2	S.	4268	1150	730		
		3	F.	1489	1050	680		
		4	F.	1118	960	680		
13	Reseeded grass after	1	S.	—	710	530	1250	760
(25. vi. 45)	old grass	2	F.	—	1960	1290		

* Total plants per plot.

It is clear from the table that marked differences in population between failing and successful plots were recorded on some of the fields. On others, the differences were quite insignificant. It is convenient to divide the fields arbitrarily into two groups on this basis: (*a*) fields 1, 2, 3, 7, 8 and 13, in which the difference in population between the failing and successful plots was not less than 530,000 wireworms per acre; (*b*) fields 4, 5, 6, 9, 10, 11 and 12, in which the difference was not more than 270,000 wireworms per acre. In spite of the apparent inconsistency of these results in relating damage to infestation, there are a number of points arising out of this table that help to clarify the problem.

It is worth noting that only three (nos. 3, 5 and 13) of the thirteen fields were freshly broken permanent pastures of the type usually surveyed for wireworm attack. Undoubtedly, this reflects the virtual cessation of the ploughing-up campaign. Nevertheless, it was surprising to find that so many of the fields showing conspicuous wireworm damage had been arable for at least 3 years. It would be useful to know whether damage on this scale in such fields is a local or occasional phenomenon, or whether it occurs more generally. At any rate, this observation underlines the fact that wireworm damage is by no means confined to newly ploughed grassland.

In about one-half of this group of fields the observed differences in the crop result coincided with marked differences in the density of wireworms in the respective areas. No case was observed in which the density of wireworms was significantly higher in the successful than in the failing area of a field. It is probably safe to say, therefore, that such a difference in infestation in different parts of a field is the most common cause of the occurrence of wireworm damage in patches.

On the other hand, there is little consistency in the levels of infestation found in areas in different fields where wireworms were actively destroying the crops. Table 1 shows that there is no consistently safe wireworm population within the wide range of populations covered here. On field 3, wheat was successful in areas with populations as high as $1\frac{1}{4}$ millions per acre (5 mm. and over). Yet in fields 4, 5 and 9, failures were observed at populations only slightly above 300,000 per acre. This furnishes additional evidence that wireworm infestation alone is not a consistent guide to the probability of damage.

The compositions of the populations do not explain these varying results at different levels of infestation. Detailed figures of larval lengths (not presented here) show that the 'low failures' do not coincide with the occurrence of a high proportion of large larvae, nor the 'high successes' with excessive numbers of small larvae, in the counts.

The plant counts (Table 1) provide a useful indication of the severity of damage. Except in field 9 there is a big reduction in the plant population on the failing plots. The plant counts in the different fields are not strictly comparable, since they reflect differences in seeding rates and stage of growth at the time of counting, but there is no evidence of more severe plant losses on fields failing at higher populations than

on those failing at lower populations (cf. fields 2 and 4). Although the reduction in plant density on the failing plots of field 9 is slight compared with other fields, the crop result on the failing area was vastly different from that on the successful area. The plants over the whole failing area appeared sickly and examination of the plants showed widespread wireworm damage. Possibly it was an early stage in the development of a failure before many of the plants had succumbed.

It is noteworthy that in group (*b*) in which crop differences were recorded without corresponding differences in population, all the fields had low populations, between 300,000 and 700,000 wireworms per acre. No case was recorded in which part of a field failed and another part was successful in a field with a uniformly high population. This might mean that in uniformly infested fields, with high populations, differences in other factors must be exceptionally great to bring about differences in the amount of wireworm damage.

History and treatment of successful and failing plots

Records of previous crops grown on the fields were obtained from the farmer, and the main points are included in Table 1. In most cases the fields had been cropped and treated uniformly. In field 9, however, although there was no difference in population between the successful and failing area, the successful area in 1945 was one on which beans had been grown in 1944. A relative immunity to wireworm attack after beans was suggested by an experiment at Rothamsted in 1941–2 and reported by Evans (1944).

With one exception, none of the fields in group (*a*), in which marked differences in population were recorded, showed corresponding differences in previous treatment. The exception was field 2, on which wheat followed trefoil on the successful area (640,000 wireworms per acre) and followed sainfoin on the failing area (1,915,000 per acre). Table 1 shows that three of the remaining fields in this group were growing cereals after sainfoin (fields 1, 7 and 8). Sainfoin is grown extensively on land adjoining the chalk in Cambridgeshire, and in 1945 a number of fields showing wireworm damage were noted on land of this type between Royston and Burwell. On most of these it was found that sainfoin had been grown recently. These observations all suggest a tendency towards high infestation and wireworm damage after sainfoin.

There is a noteworthy distinction between the fields in group (*a*), where patches were associated with differences in population, and those in group (*b*), where no such differences were detected. The failing crops in group (*a*) followed either sainfoin or grass, whereas, with the exception of field 5, all the fields in group (*b*) had a long arable history. This suggests that uneven wireworm distribution with areas of heavy infestation commonly occurs after grass or forage crops, but after successive years of cropping uniform distribution is the rule.

Soil analysis

The results of the soil analyses* are not presented here in full. Although the fields covered a wide range of soil conditions, none of them was deficient in any of the chemical factors investigated, with the exception of field 5, which was deficient in lime. There is no evidence that any of these constituents were consistently low or high on those plots which failed at low populations.

The differences between fields have less bearing on the problem under discussion than the differences within fields, and the latter are set out in full in Table 2. It is clear that big differences exist between failing and successful plots on most fields with respect to one or more of the factors investigated. In only three of these, however, is there any consistent association with successful or failing crops: $CaCO_3$, coarse sand and fine sand.

With the solitary exception of field 12, the failing plots show a higher $CaCO_3$ content than the successful plots. In some cases, e.g. nos. 5 and 13, the difference is insignificant. In field 2 it is small (3·31%) compared with the $CaCO_3$ content of the field (65%). Yet in those fields where marked differences in $CaCO_3$ were detected (nos. 1, 3, 6, 9, 10 and 11), the excess always appeared in the failing plots. Moreover, the fields with the largest differences in $CaCO_3$ (nos. 6, 9, 10 and 11) proved to be fields in which failure had occurred at low populations and without marked differences in population between the failing and successful plots. There is more than a suggestion here that wireworm damage tends to occur on areas within a field which are higher in $CaCO_3$ and which often do not contain higher wireworm populations.

The other chemical constituents in Table 2 throw no light on the problem: pH is comparatively uniform within each field. Loss on ignition and organic carbon, being a measure of the organic matter in the soil, should reflect any excess of plant matter in the soil (e.g. decaying turf in newly ploughed grass fields) which might serve as an additional source of food for the wireworms and minimize the damage to the crop. Although these factors are higher in those fields (nos. 3, 5 and 13) recently ploughed from grass, no relation between them and the incidence and severity of wireworm attack is apparent.

Conspicuous differences in the sand, silt and clay fractions of the soils were recorded between the successful and failing plots in many fields. The differences in silt and clay show no special association with the crop results observed. The differences in sand content, however, show a definite tendency for high sand content to be associated with the successful plots. This association is particularly conspicuous in the coarse sand fraction where it is true of every field except field 8. In four cases the differences were insignificant, but in the remaining nine the differences were sharp and in three of these (nos. 5, 9 and 13) very marked indeed.

* Carried out in the Soil Science Laboratory, School of Agriculture, University of Cambridge, through the kindness of Mr F. Hanley.

The fine sand fractions follow a parallel trend, although the differences are smaller and the trend is less consistently in support of an excess in the successful plots. Only field 1, however, shows a significant excess of fine sand in the failing plots.

Table 2. *Differences between successful and failing plots in populations and soil factors. Figures represent* F.–S. *Averages of paired plots taken*

Field no.	Wireworm population: All sizes	Wireworm population: 5 mm. and over	pH	$CaCO_3$	Available P_2O_5	Available K_2O	Coarse sand	Fine sand
1	+ 900	+ 910	+0·09	+ 8·47	+0·007	0·000	− 0·76	+ 5·71
2	+1495	+1275	0·00	+ 3·20	+0·020	+0·001	− 4·36	− 0·20
3	+2285	+1390	+1·20	+ 3·31	+0·007	+0·007	− 9·40	−11·58
4	+ 260	+ 180	+0·17	+ 1·50	−0·001	+0·007	− 1·55	− 4·13
5	+ 40	+ 40	−0·22	+ 0·13	−0·001	+0·006	−17·65	− 3·67
6	+ 30	+ 10	0·00	+30·32	+0·007	+0·012	− 2·73	− 6·73
7	+ 800	+1080	−0·07	+ 1·07	+0·016	+0·016	− 0·07	+ 1·13
8	+ 600	+ 530	−0·08	+ 2·65	−0·017	−0·017	+ 6·74	− 8·40
9	+ 180	− 10	+0·09	+15·37	+0·050	−0·003	−11·98	− 2·50
10	+ 180	+ 270	+0·12	+11·93	0·000	+0·007	− 7·56	− 3·00
11	+ 30	+ 30	+0·12	+13·93	+0·004	+0·002	− 9·30	− 3·19
12	+ 5	+ 100	−0·06	− 0·60	+0·002	−0·007	− 0·37	+ 0·77
13	+1250	+ 760	−0·16	+ 0·02	−0·013	−0·004	−18·15	− 0·44

Field no.	Silt	Clay	Loss on solution	Loss on ignition	Total nitrogen	Moisture	Organic carbon
1	−1·94	−10·84	+0·02	−0·53	−0·140	−1·31	+0·16
2	−0·55	+ 1·49	+0·97	−0·45	−0·006	+0·31	0·00
3	+1·90	+13·58	−0·18	+3·74	+0·120	+1·37	+0·62
4	+1·13	+14·38	−0·10	+1·30	+0·003	+0·59	−0·29
5	+0·30	+17·46	−0·63	+1·82	+0·028	−0·30	+0·50
6	−5·13	−14·10	−0·52	−1·43	+0·049	−2·54	+0·46
7	0·00	− 0·70	+0·35	+0·24	+0·036	−0·12	+0·14
8	−3·65	− 1·46	−0·27	−1·74	+0·010	+2·33	0·00
9	−0·18	− 0·04	−0·08	−0·42	−0·011	−0·04	0·00
10	+3·83	+ 0·94	−0·37	−1·74	−0·070	−2·75	−0·47
11	−1·09	+ 0·85	+0·44	+0·39	−0·040	−0·27	−0·16
12	+0·12	− 0·57	+0·58	+0·37	+0·005	−0·12	+0·14
13	+5·27	+ 4·80	+1·64	+7·14	+0·183	+3·51	+0·79

Wireworm activity

Table 3 shows the results of tests carried out by Dr Crombie on the biting responses of wireworms collected from the failing and successful plots in fields 1, 2, 3, 4, 6 and 9. For each test twenty wireworms were placed on filter-paper moistened with 2% glucose, and the number of bites after 24 hr. at 25° recorded. Tests with filter-papers moistened with water only served as controls. The main features of this method were outlined by Thorpe, Crombie, Hill & Darrah (1945).

The results of these tests were inconclusive. There are cases, such as fields 1, 4 and 6, where a marked difference in the number of bites was recorded with larvae from the damaged and undamaged plots. However, wireworms from failing plots might feed more actively simply because they have been collected from an environ-

ment that is deficient in food as a result of the reduction in its plant population. For this reason, no special significance can be attached to differences of this kind.

TABLE 3. *Biting responses of wireworms from successful and failing plots*

Figures represent no. of bites on filter-papers moistened with water and 2% glucose by twenty wireworms in 24 hr. averaged from four or six separate tests.

Field no.	Successful		Failing	
	Water	2% glucose	Water	2% glucose
1	20	283	40	537
2	10	152	12	225
3	10	220	11	210
4	5	43	5	200
6	15	323	20	567
9	5	252	15	278

TABLE 4. *Wireworms collected from successful and failing plots*

Figures represent number collected per man per hour. Average of two where pairs of plots examined.

Field no.	Successful	Failing
1	19	75
2	32	93
3	47	100
4	7	20
5	3	21
6	32	49
7	3	28
8	10	23
9	23	47

Table 4 shows the rate of collection of wireworms by the field workers on the failing and successful plots, expressed as wireworms per man per hour. In general, these figures follow the estimates of population as would be expected (cf. Table 1), but the relationship is not a close one. There is one marked difference between these two sets of results. In every case, larvae were found more readily on the failing plots. Even fields such as nos. 4, 5, 6 and 9, where differences in population between the plots were not detected in the samples, showed clear differences in the rate at which larvae could be collected from the failing and successful plots. This suggests that real differences do exist in the behaviour and orientation of the larvae in the different plots.

DISCUSSION AND CONCLUSIONS

The results of this investigation reflect its exploratory and tentative character. Yet they have confirmed that other factors besides infestation play an important part in determining the amount of wireworm damage in a given case. This is shown clearly by two observations: (1) only six out of the thirteen fields showed significant differences in populations between failing and successful plots; (2) the populations on the failing plots varied between wide limits in the different fields.

The results of the tests on other factors present us with data of a new type. Most of these factors have been considered previously only as factors which might influence the density of a wireworm population. Thus soil analyses were taken throughout the Wireworm Survey and the results correlated with the estimates of wireworm population, but no significant correlations were detected (Advisory Entomologists' Conference, 1944). Recently, Salt & Hollick (1946) have published results of an intensive quantitative study of a variety of factors on two grass fields, in which they correlated these factors with the number of wireworms found at selected spots. In one field significant positive correlations were detected between the wireworms collected, the organic content (loss on ignition) of the soil, the prevalence of the grass *Lolium* and the number of staphylinid larvae. In the other

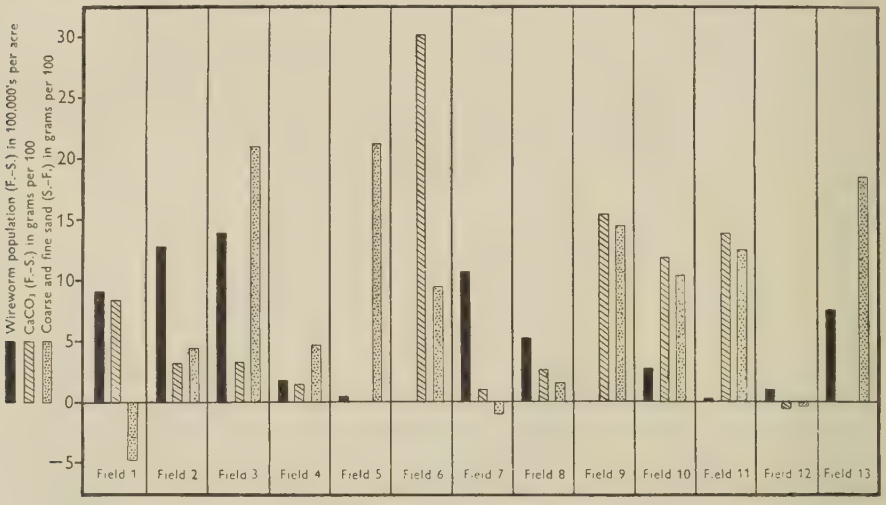

Fig. 1. Differences between failing (F.) and successful (S.) plots in fields 1–13.

field a negative correlation with moisture was detected. In the fields studied in this investigation, no relation was evident between the density of wireworm populations in the different fields and the other factors examined.

More positive conclusions can be reached with regard to differences between successful and failing plots within the fields. Besides wireworm population, $CaCO_3$, sand and a sainfoin history showed differences within fields that were frequently associated with the success or failure of a crop. There is clearly a tendency for failures to occur on those parts of a field that are rich in $CaCO_3$ and poor in sand.

In Fig. 1 the differences between the failing and successful plots for each of the factors, infestation, $CaCO_3$ and sand content, are presented in a form which shows the distribution and the magnitude of the differences. There are several noteworthy points in the diagram. First, there is only one case where one of these factors shows

a significant difference opposing the general tendency, the excess sand in the failing plot in field 1. In all other cases the differences are either insignificant or follow the above-mentioned trends. Secondly, on most fields in the series, marked differences were recorded in one or other of these factors. Field 12 alone showed no differences in infestation, $CaCO_3$ or sand between the failing and successful plots. Thirdly, in some cases (fields 1, 3 and 13), marked differences in $CaCO_3$ or sand occurred in conjunction with big differences in population. In general, however, the differences in sand or $CaCO_3$ are most conspicuous on those fields where differences in infestation were not recorded, and of these, fields 5, 6, 9, 10 and 11 are outstanding.

When examined in this way, these results strongly suggest that these three factors are jointly responsible for determining whether or not wireworm damage will occur in a given case. But although the factors $CaCO_3$ and sand seem to account for some of the differences in crop results within single fields that cannot be explained by differences in populations, they do not explain the differences between fields so satisfactorily. Table 5 shows the results of the tests on these three factors alone. In

TABLE 5. *Wireworm populations*, $CaCO_3$ *and sand content of successful and failing plots*

	Successful			Failing		
Field no.	Population 5 mm. and over	$CaCO_3$ %	Sand %	Population 5 mm. and over	$CaCO_3$ %	Sand %
1	540	3·29	30·47	1450	11·75	35·42
2	640	63·00	11·92	1915	66·21	7·41
3	1245	0·30	38·46	2635	3·61	17·48
4	145	1·30	21·67	325	2·80	15·98
5	290	0·67	58·01	330	0·80	36·70
6	690	23·58	27·38	700	53·90	17·92
7	530	23·87	41·76	1610	24·94	42·82
8	980	35·36	44·18	1510	38·01	42·52
9	345	40·29	39·35	335	55·67	24·87
10	310	23·43	36·48	580	35·36	25·93
11	320	24·97	41·75	350	38·90	29·26
12	580	7·79	59·26	680	7·19	59·66
13	530	0·17	67·99	1290	0·19	49·40

field 3, low $CaCO_3$ and high sand content coincide with the occurrence of a good crop at a high wireworm population, as the evidence presented so far might lead one to expect. In field 13, however, a failure occurred under identical conditions. Moreover, a high $CaCO_3$ content is not invariably associated with crop failure from wireworms and on the successful plot in field 2, $CaCO_3$ is higher than on any of the failing plots in the other fields. Again, in field 5, $CaCO_3$ is very low and sand very high, yet a failure occurred at a low population. In this table, the successful parts of fields 3 and 8 might rank as 'high successes' and the failing parts of fields 4, 5, 9 and 11 as 'low failures'. The data show that these cannot be explained con-

sistently by assuming that wireworm damage is more severe in soils rich in $CaCO_3$ and poor in sand. It is evident that the relationship disclosed in this comparison of failing and successful plots accounts quite well for differences within fields but very poorly for differences between fields.

Wireworm damage must be the outcome of a number of environmental conditions exerting their effects through wireworm density on the one hand and wireworm activity on the other. So far, the main emphasis has been on wireworm density, on populations, and there has been a tendency to regard environmental factors as important only in so far as they affect the number of wireworms inhabiting the soil. It has been assumed for practical purposes that a number of wireworms in any given field will do as much damage, on the average, as the same number of wireworms in any other field. Yet, arguing on *a priori* grounds, a severe wireworm attack is as likely to reflect a small number of wireworms feeding very actively as a large number feeding at an average rate. If the range of populations found in fields is considered in relation to the number of plants in a cereal crop, the importance of this aspect of the problem will be obvious.

In its young stages the plant density of a cereal crop is of the order of 1–1½ millions per acre. A reduction of this figure by ½–⅔ would be classed a wireworm failure for our purpose, i.e. a loss of ½–1 million plants. Our records of fields sampled with the washing and flotation method show that very few fields, even those classed as old arable, contain less than 250,000 wireworms per acre. Therefore, in a field with a very low population, it is only necessary to assume that each wireworm destroys two to four plants in order to bring about an utter failure of the crop. At the other end of the scale, with a population of 2 millions per acre, if one wireworm in every two to four should attack and kill a plant, a failure will result.

These computations can be related to Broadbent's (1946) figures on the incidence of wireworm attack on young plants grown in pots. He found that six larvae of *Agriotes* destroyed twenty-seven out of 150 oat plants when one larva was placed with twenty-five plants in each pot, a density corresponding to 125,000 per acre. Thus each larva, at this density, is capable of destroying four to five plants. Comparing these figures with those above, it will be seen that almost every field contains enough wireworms to cause a failure of a cereal crop, if they are feeding at what Broadbent's figures suggest is a normal rate. When the problem is considered in these terms, the failures observed at low populations occasion no surprise. What is surprising is that so many cereal crops survive in the presence of moderate and high populations where there is at least one larva to every plant. These points underline the decisive part which factors influencing locomotory and feeding activity of the wireworms must play in determining the amount of damage that occurs. Probably it is through such an action on behaviour that $CaCO_3$ and sand content of the soil affected the severity of wireworm damage in the fields under review.

In conclusion, a few remarks should be made about the prediction of wireworm damage in the light of these observations. We now have some evidence that $CaCO_3$

promotes and sand inhibits wireworm damage in the fairly uniform conditions within a single field. It is highly probable that these factors act in the same way in different fields, and that data on them, together with data on infestation, would assist the prediction of the crop results. However, the exact relation is not clear, and another factor or factors, varying from field to field but apparently uniform within most fields, seems to influence the final crop result. This factor might be revealed by continuing studies on failing crops, concentrating on areas where failures are occurring at low populations and successes at high populations, and testing factors such as moisture, which were not included in this investigation.

One of us (Ross, 1946) has already pointed out that the discovery of the effectiveness of benzene hexachloride against wireworms (Thomas & Jameson, 1946) does not remove the need for predicting in what fields wireworm damage is likely to occur. This is because this insecticide must be applied with the seed, that is, before it is known whether an attack will develop. Unless it is intended to treat every field containing a potentially dangerous population (this means in practice the majority of fields containing appreciable numbers of wireworms), the most effective use of such a treatment depends on reliable predictions about the probability of wireworm attack. Therefore it would be wrong to believe that the need has passed for field and laboratory studies which aim to provide a sounder basis for predicting wireworm damage.

The authors have received assistance and advice from many quarters during the course of this investigation. The work was carried out in the Advisory Entomology Department at the School of Agriculture, Cambridge. We are grateful to Mr F. R. Petherbridge, head of this department, for his support and encouragement at all times. We are particularly indebted to Mr F. Hanley, Advisory Chemist at the School of Agriculture, for arranging the analysis of soil samples in his laboratory and for his advice on the presentation of the results. We thank Dr R. K. Schofield and Dr A. C. Evans, of the Rothamsted Experimental Station, for their advice, and regret that we could not carry out all their valuable suggestions. Dr W. H. Thorpe and Dr A. C. Crombie, of the Zoological Laboratory, Cambridge, obliged us by carrying out the tests on the feeding activity of the wireworms collected. We also thank Dr J. Wishart for advice on the statistical aspects of the investigation and Dr N. H. Pizer for advice on results of the soil analyses. We can only thank collectively the farmers, whose land we used for these experiments, for their co-operation and friendly interest. Finally, our gratitude is due as before to our laboratory staff for their co-operation in a very strenuous investigation.

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(*Received* 30 *September* 1947)

THE USE OF METALDEHYDE AGAINST SLUGS

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(With 7 Text-figures)

One part of metaldehyde in thirty parts of bran by volume is the minimum effective concentration for obtaining an economic kill of slugs under average weather conditions. For use against *Milax gracilis* Leydig. heaps of bait should not be normally more than 2 yd. apart and against *Arion hortensis* Fer. not more than 3 ft. apart. The mortality of slugs attracted to metaldehyde-bran baits is dependent on the drying property of the soil and atmosphere around the baits. D.D.T. flea-beetle dust and powdered pyrethrum flowers are sufficiently distasteful to slugs to prevent their consuming a lethal dose of the poison mixed with bran. The addition of 10%, by volume, of dried blood to metaldehyde-bran bait increases the catch of some slugs under certain conditions but does not increase the mortality. The addition of 10%, by volume, of dextrose increases the mortality produced by metaldehyde but does not increase the numbers attracted to the bait. A metaldehyde-bran bait compacted into 'biscuit' form with anhydrous calcium sulphate catches more slugs than an ordinary metaldehyde-bran bait, but almost completely counteracts the effect of the metaldehyde. A metaldehyde-bran-casein glue bait in 'biscuit' form increases the catch and kill of *Milax gracilis* and *Agriolimax reticulatus* Müll., but not of *Arion hortensis*. Metaldehyde-bran-casein glue bait broadcast in 'broken biscuit' form is effective over a longer period, thus producing a greater kill of slugs, than a broadcast metaldehyde-bran bait.

Introduction

Barnes & Weil (1942) investigated a wide range of attractants used with metaldehyde and reached the important conclusion that metaldehyde has itself only small powers of attracting slugs compared with those of bran, and that the higher the nutritive qualities of the attractant the greater was its attraction to slugs. These experiments did not discover the effects which differences in the physical form of the attractant might make in the desiccation of slugs partly immobilized by metaldehyde, nor did they indicate if there were any differences in the preferences of various species of slugs for different attractants.

One of the main difficulties in determining the efficiency of any method of baiting for slugs is the problem of estimating the number of slugs present on the experimental plots. A solution of this problem was suggested by Thomas (1944) using standardized metaldehyde-bran baits to determine the relative population of slugs on treated and control plots before and after the treatment under investigation. Some refinements of this technique were found to be necessary and are described in this paper under the section dealing with 'broken-biscuit' bait.

Very little consideration has hitherto been given to the actual killing quality of a poison bait apart from its ability to attract slugs, or to the climatic factors

influencing the mortality of metaldehyde-poisoned slugs. Gimingham & Newton (1937) were amongst the earliest workers to point out the disadvantages of broadcast metaldehyde-bran bait, while Barnes & Weil (1942) remarked on the deterioration of heaps of bait by mould and bacterial fermentation.

The action of metaldehyde on slugs and factors affecting it

The physiological details of the action of metaldehyde on slugs and snails still require investigation, but a few useful general observations have been made in the course of many experiments with metaldehyde against slugs. The effects of metaldehyde appear to be threefold. There is a direct mortally toxic effect producing a characteristic transparency of the gut wall. A slug suffering from this effect cannot be restored by washing it in water or by preventing desiccation by confining it in a water-saturated atmosphere. Secondly, there is an anaesthetic effect under which a slug can, but will not, move, unless exceptionally stimulated. Thirdly, there is an irritant effect causing the excessive production of mucus which increases the desiccating effect of a dry atmosphere around the slug. Both these effects can be counteracted by washing or confining the slugs in a saturated atmosphere, conditions which are brought about in the field by rainfall and absence of wind and sun. It seems likely that the second effect is merely a mild form of the first, when an insufficient quantity of bait has been eaten to cause total paralysis and death. This is supported by the fact that of any catch of slugs by metaldehyde-bran baits only a small proportion will die if washed with water, provided that they have not been previously exposed to a drying atmosphere, even though the slugs have not produced excessive mucus nor shown any tendency to move to cover. The third effect is possibly due to some contact action of the metaldehyde or dry bran on slugs partially poisoned by metaldehyde. In experiments involving 293 *Milax gracilis* only 29% died after 72 hr. when the metaldehyde-poisoned slugs were removed from the bait (itself a dehydrating agent when dry) and kept in a saturated atmosphere. 240 *M. gracilis* caught at the same time on similar baits showed 87% deaths after exposure to desiccation in a dry flower-pot for 72 hr. 100 healthy slugs all survived 72 hr. of exactly similar treatment.

The results of a similar experiment under field conditions are summarized in Table 1. Freshly prepared baits containing 1 vol. of metaldehyde in 10 vol. of bran were used. This bait will be referred to as the 'standard' bait. Fifty traps were exposed at night, each consisting of 1 teaspoonful of bait covered with a 6 in. square of glass painted black on the upper surface. The catch was counted at 9 a.m. the following morning. The mortality was calculated from the number of dead slugs remaining around the baits at 4 p.m. on the same day. The glass covers were removed from the baits at the morning count, so that the catch was exposed to normal atmospheric conditions from then until the mortality count. Fresh bait in new positions was used each day. The night or day was considered dry if no rain fell. A warm day was one with a maximum temperature of 65° F. or over, and a cold

day one with a maximum of 64° F. or less, and a cold night one with a minimum of 40° F. or less.

TABLE 1. *The effects of weather conditions on the catch and mortality of* Milax gracilis *at metaldehyde-bran baits*

Weather		No. of trials	Mean catch per day	Mean % mortality
Night	Day			
Wet and cold	Wet and warm	7	170± 43	22±10
Wet and warm	Wet and warm	10	531±109	35±14
Wet and warm	Dry and warm	2	470± 91	82±12
Dry and cold	Dry and warm	5	43± 17	98± 2
Dry and cold	Dry and cold	7	121± 37	76±17

Wet and warm weather produced greater catches but lower mortality; cold dry nights followed by warm dry days produced the smallest catch with the highest mortality. This increase in mortality did not, however, counterbalance the fall in the numbers caught. Wet warm nights followed by dry warm days are apparently the ideal conditions for obtaining a maximum catch with maximum mortality.

Barnes & Weil (1942) found that rain after dry periods increased the number of slugs caught by metaldehyde-bran baits. It has been observed, however, during 5 years' experience of slug baiting in Devon, that if the ground is already suitably damp for slugs most species tend to be less active during heavy rain. *Arion hortensis* appears to be exceptional in that it is sometimes extremely active during rainstorms, even in the daytime.

The desiccating property of the air around the baits is affected by local air temperature and air movement, the quantity of water on the soil surface and on vegetation around the baits, and also by the shelter afforded by such vegetation. The nature of the soil surface is also important—for instance, the rough surface of a field of wheat in winter gives far more protection from sun and drying winds than the compact and level surface of a *Brassica* seed-bed. These are important factors to be borne in mind by the research worker and by growers attempting practical control in the field.

THE ACTION OF METALDEHYDE ON SNAILS AND ON THE EGGS OF SLUGS

Some snails, particularly the garden snail, *Helix aspersa* Müll. (the European brown snail of American authors), are apparently more susceptible than slugs to the direct toxic action of metaldehyde. On eating a bait containing metaldehyde they die rapidly, contracting, with much effervescence of mucus, into their shells. Conditions of high relative humidity have little or no effect in preventing or even delaying death. These symptoms, observed by the author in Devon gardens, are not confirmed by Persing (1944) in California, who states that the metaldehyde paralyses the snails on contact, usually with their bodies extended from their shells, and that they then die from the effects of desiccation of the exposed parts. Preliminary

experiments in Devon suggest that metaldehyde, when placed in contact with the bodies of healthy active snails, is merely sloughed off with the mucus, as is any inert dry powder such as finely ground limestone. No lethal effects were noticed as a result of such contact with metaldehyde, and it is suggested that with snails metaldehyde acts entirely as a stomach poison.

Precise experiments on the effect of more prolonged contact with metaldehyde on slugs or snails are difficult, as it is almost impossible to prevent them from eating particles of metaldehyde. Jary & Austin (1937) described an experiment in which slugs and snails had access, overnight, to heaps of a mixture of metaldehyde and dry soil, and attribute the resultant deaths to the contact effect of metaldehyde. In the absence of evidence that metaldehyde had not been eaten such an experiment cannot be regarded as conclusive.

Lange & MacLeod (1941) reported that a talc dust containing 10% of metaldehyde completely prevented the hatching of the eggs of the field slug, *Agriolimax reticulatus*. Eggs of slugs are particularly susceptible to desiccation, and an experiment showed that a dust of basic slag was equally toxic to the eggs of both *A. reticulatus* and *Arion hortensis* whether it contained 10% of metaldehyde or not. Undusted eggs laid at approximately the same time as the treated eggs had a viability ranging from 73 to 92% per batch of eggs.

The minimum effective quantity of metaldehyde in bran baits for slugs

An attempt to discover the best concentration of metaldehyde in bran for use against slugs was made by comparing baits of strengths varying from 1 vol. of metaldehyde in 10 vol. of bran to 1 vol. of metaldehyde in 50 vol. of bran. Twenty-five replicates of each strength of bait were used, each replicate being 1 teaspoonful of bait placed on the soil under a blackened glass plate. The baits were placed 10 yd. apart in ten rows of five baits per row. This distance apart was considered sufficient to prevent interference between adjacent baits (vide next section). In this experiment *Milax gracilis*, *Agriolimax reticulatus* and *Arion hortensis* were caught in sufficient numbers for the results to be examined statistically. In the second experiment strengths of baits from 1 in 25 to 1 in 200 and twenty-five replicates of each strength were used; only *Milax gracilis* was present in sufficient numbers.

The mortality of slugs was determined by collecting slugs separately from each bait strength and placing equal numbers of them in 10 in. flower-pots, without drainage holes, containing as nearly as possible the same quantity of dried hay in each. The pots were covered with earthenware saucers and the surviving slugs counted the following morning. This technique, though artificial, has the advantage of eliminating the possibility of slugs reaching cover and subsequently dying. The desiccating quality of such a hay-pot under the conditions of these two experiments was approximately equivalent to that of a dry day on a damp soil with a wind of Beaufort Scale force 2 (i.e. 5 m.p.h.) and a maximum temperature of 65° F. The

results (Table 2) suggest that a concentration of 1 vol. of metaldehyde to 30 vol. of bran is the minimum effective dose under these rather stringent conditions. The figures for the actual number of slugs attracted to the different strengths of bait confirm the conclusions of Barnes & Weil (1942) that metaldehyde itself has relatively little power of attraction compared with that of bran. There were no significant differences in the number of slugs caught at the different strengths of bait, even between the catch of the 1 in 10 baits and the 1 in 200 baits after allowing for differences in the activity and population levels in the two experiments.

These results do not preclude the possibility of a metaldehyde-bran bait in the proportions of 1 in 100 producing a mortality sufficiently high to be economically useful, provided that the weather conditions were exceptionally favourable for high mortality. But it must be remembered that such conditions (see Table 1) are rarely accompanied by conditions favourable for high-percentage nocturnal slug activity and hence for a large catch per trap or unit area baited.

TABLE 2. *The efficiency of different strengths of metaldehyde-bran baits against slugs. Twenty-five replicates of each bait strength*

	Milax gracilis		*Agriolimax reticulatus*		*Arion hortensis*	
Metaldehyde/bran strength	No. caught	% killed	No. caught	% killed	No. caught	% killed
1/10	602 ± 49	89 ± 6·2	307 ± 33	91 ± 8·1	181 ± 17	84 ± 9·0
1/20	541 ± 44	87 ± 7·1	290 ± 30	87 ± 8·1	207 ± 19	87 ± 10·2
1/30	621 ± 61	80 ± 5·3	331 ± 28	88 ± 8·0	199 ± 20	71 ± 5·7
1/40	580 ± 38	62 ± 4·8	311 ± 35	73 ± 6·9	220 ± 19	56 ± 4·2
1/50	593 ± 38	54 ± 4·2	287 ± 25	59 ± 4·2	184 ± 27	53 ± 5·0
1/25	135 ± 19·6	91 ± 8·6				
1/50	113 ± 14·7	52 ± 4·7				
1/100	126 ± 17·0	16 ± 2·8				
1/150	92 ± 18·5	20 ± 3·1				
1/200	129 ± 20·2	10 ± 1·8				

TABLE 3. *Numbers of slugs caught and percentage killed on metaldehyde-bran baits covered by night and by day and on baits covered only at night. Fifty replicates of each treatment*

	Covered baits		Exposed baits	
Species of slug	Catch	% mortality	Catch	% mortality
Agriolimax reticulatus	266	46 ± 12	252	91 ± 15
Arion hortensis	127	27 ± 7	142	73 ± 13
Milax gagates	51	33 ± 14	70	79 ± 16

THE MAXIMUM EFFECTIVE DISTANCE BETWEEN BAITS

It has been shown by Thomas (1944) that covered baits catch more slugs than uncovered baits, but further experience has shown (Table 3) that the covers protect the slugs from desiccation, and the number of slugs killed is actually less than on uncovered baits. The best kill is obtained by covering the baits during the night and

removing the covers in the morning to expose the catch. As a practical control measure this could only be done on horticultural holdings where the expense of the extra labour was commensurate with the gains due to the control of the slugs. The disadvantages of metaldehyde bran as a broadcast bait are discussed in a later section of this paper.

The optimal distance between baits is approximately the average distance at which a slug is attracted to the bait in the course of its supposedly random wanderings in search of food. This distance will probably vary from one species to another and with the size of the individual slugs. The roughness of the soil surface, the distribution and quality of other available food and the amount of humidity both in the atmosphere and on the soil surface are other factors likely to affect the range of attraction of the bait.

The method adopted by Thomas (1944) of using circles of ammonium sulphate of various diameters to determine the range of attraction of metaldehyde-bran bait for *Agriolimax reticulatus* is open to the objection that the ammonium sulphate may go into solution in the soil surface film of moisture and thus influence the behaviour of the slugs within the enclosing barrier. These barriers are, moreover, undoubtedly less effective against such subterranean slugs as the species of *Milax* than against a surface species such as *Agriolimax reticulatus*.

A different technique was therefore adopted to measure the effective range of the traps. Two species of slugs of major importance in horticulture, *Milax gracilis* and *Arion hortensis*, were selected. Groups of twenty-five 'standard' baits were placed on suitable ground in a 5×5 lattice square. The distance between the traps in each direction was the same within a group, but there were different distances for each group. The central nine baits of each group were therefore liable to be influenced by the presence of traps all round them. The remaining sixteen traps in each group formed an outer ring, the catch on which was not comparable to that on the inside block. In making the counts of slugs only this inside block of nine traps was examined. Where the count on any one block was significantly lower than on other blocks it was considered that these baits had been closer than the range of the attraction of the bait, and that the distance between the baits in this block was less than the minimum distance from the optimum catch. The ground for this had been shown by previous sampling to have a very uniform population of both species of slugs. The plot was a bare fallow following a crop of onions and the soil surface was flat and compact. There was no cover other than that afforded by the glass covers on the baits. One or two slight showers of rain occurred during the night, the maximum temperature of which was 56° F. and the minimum 44° F. The weather conditions were therefore slightly above the average for slug activity.

The results, expressed diagrammatically in Fig. 1, can only be used as a rough guide for the spacing of the traps under the particular conditions of the experiment. The baits spaced 1 ft. apart had below the average numbers of both species; those 2 ft. apart showed mutual interference for *Milax gracilis* but not for *Arion hortensis*,

indicating that the latter had a smaller range than the former species. At 3 ft. and more apart there was no interference for either species. It is concluded that in baiting against *Milax gracilis*, under such conditions as these, the baits should not be farther than 1 yd. apart and against *Arion hortensis* not more than 18 in. apart.

In practice, where the baits are left on the ground for more than one night the spacing can be increased somewhat as slugs will gradually migrate from the intervening areas into the radius of attraction of the baits. It is probable that the maximum distance between baits under weather conditions in which they could be left down without going mouldy or sour for four or five nights, is about 6 ft. for *Milax gracilis*, and rather less for *Arion hortensis*.

Special spacing and arrangement of baits may have to be used to prevent the migration of slugs into an area containing a highly attractive crop such as strawberries or lettuce. A barrier of baits not more than 1 ft. apart might have to be used to check the invasion of the area (assuming that it had already been cleared of slugs) by such species as *A. hortensis*.

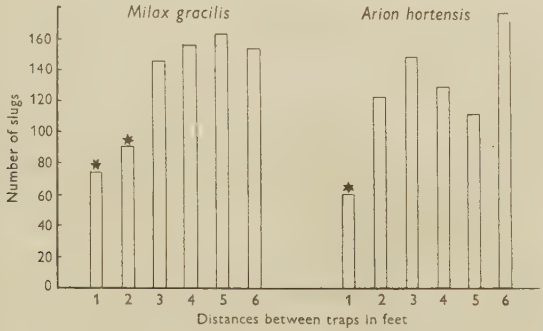

Fig. 1. The range of attraction by metaldehyde-bran bait. Catch of slugs on traps at different spacings. * denotes significant difference at $P=0{\cdot}01$ level.

The efficiency of metaldehyde and bran baits compared with that of other poisons and attractants

In this experiment 'standard' metaldehyde-bran bait was compared with the following:

1 vol. metaldehyde in 10 vol. of fine acorn flour.

1 vol. 5% D.D.T. flea-beetle dust in 10 vol. of bran.

1 vol. powdered pyrethrum flowers in 10 vol. of bran.

1 vol. metaldehyde in 1 vol. of dried blood and 10 vol. of bran.

Twenty-four replicates of each type of bait were randomized on a 3 yd. lattice on land carrying a crop of tomatoes. Each replicate consisted of 1 teaspoonful of bait placed on the soil and covered with a 6 in. square blackened glass plate. The traps were examined at 9 a.m. to count the catch and again at 3 p.m. to determine the

mortality. This method of estimating mortality is not so precise as the 'hay-pot' technique but gives a valid relative figure for different baits for the one experiment. Only *Milax gracilis* was caught in sufficient numbers to provide data suitable for statistical analysis.

TABLE 4. *Comparison of numbers of* Milax gracilis *caught and killed by various poison baits. Twenty-four replicates of each type of bait. * indicates a significant difference from 'standard' bait*

Bait	Average no. caught per trap	Average % mortality
Metaldehyde and acorn flour	23·5± 4·1	66·0±8·5
Metaldehyde and bran plus dried blood	30·8±10·5	79·2±6·7
D.D.T. and bran	11·2± 3·5*	0·0*
Pyrethrum and bran	6·5± 2·5*	0·0*
'Standard' metaldehyde and bran	31·0± 4·7	85·9±9·1

The results (Table 4) show that while 'standard' bait was much superior to both the D.D.T. and pyrethrum baits, it was not, under the conditions of the experiment, better than the acorn flour or dried-blood baits. On this particular plot the *M. gracilis* had been feeding on overripe windfall tomatoes and, in the light of later experiments, it seems likely that this may have somewhat altered their preferences for the different baits, particularly for the dried-blood bait. This emphasizes the importance of having some knowledge of the previous food of slugs on experimental plots before interpreting results.

In another experiment fifty baits of metaldehyde, dried blood and bran were compared with fifty 'standard' baits. The baits were randomized in a 10 × 10 block on a piece of horticultural land known to be heavily infested with both *Arion hortensis* and *Milax gracilis*. The baits were kept in the same position for nine consecutive nights and the catches counted and removed each morning. The numbers caught (Figs. 2, 3) show that the dried-blood baits were consistently more attractive to *M. gracilis* than were the 'standard' baits—the total numbers caught being 955 and 594 respectively, a highly significant difference. *Arion hortensis* was not attracted to one bait more than another.

Metaldehyde-bran baits containing sugar have been used with success against both slugs and snails by Lange & MacLeod (1941) in California. For many years it has been the custom in this country to add molasses to poison baits of bran and Paris green used against cutworms (*Agrotis* spp., Lep.) (Fryer & Stenton, 1923) and leatherjackets (*Tipula* spp., Dipt.). It has been generally assumed, though direct confirmation seems to be lacking, that the molasses increases the attractiveness or palatability of the bait to these pests. In order to get more precise information experiments were made comparing 'standard' bait with similar bait containing in addition 10% by volume of dextrose. In the first experiment thirty traps of each bait were used, 1 teaspoonful of bait to each trap. The mortality was measured in

the field as described for the previous experiment. In the second experiment fifty-six replicates of each bait were compared and the mortality measured by the 'hay-pot' technique. The results (Table 5) indicated that, while there was no significant increase of catch of *Milax gracilis* on the 'sugar' baits over the 'standard' baits, there was a significantly consistent increase in the mortality on the 'sugar' baits. A practical disadvantage of using sugar in the bait is that it tends to go mouldy more rapidly than the 'standard' bait.

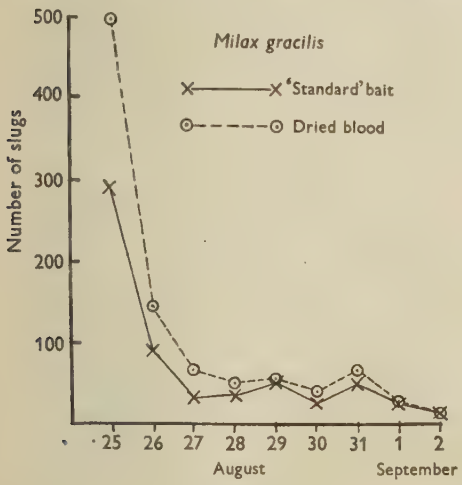

Fig. 2.

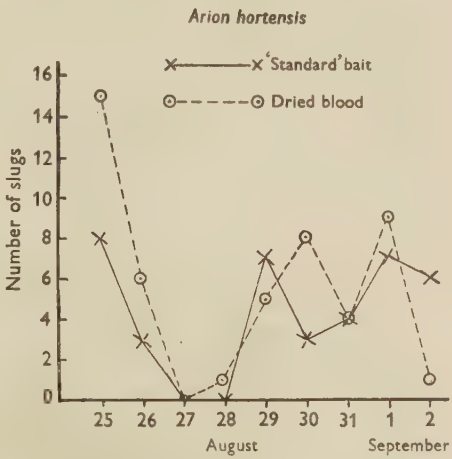

Fig. 3.

Figs. 2, 3. Comparison of the catch of slugs by 'standard' bait and by a metaldehyde bran containing dried blood.

TABLE 5. *Numbers of* Milax gracilis *caught and killed by 'standard' metaldehyde-bran bait and similar bait containing* 10% *by volume of dextrose*

		No. caught	% killed
19–20 Oct. 1944, 30 replicates:	'Standard'	171 ± 24·6	46·2 ± 4·8
	Dextrose	178 ± 20·6	60·1 ± 6·2
23–24 Oct. 1944, 56 replicates:	'Standard'	109 ± 20·2	38·0 ± 6·3
	Dextrose	125 ± 14·1	58·0 ± 8·1
	Healthy (control)	100	0·0
24–25 Oct. 1944, 56 replicates:	'Standard'	107 ± 17·6	57·0 ± 6·0
	Dextrose	109 ± 21·1	72·0 ± 7·2
	Healthy (control)	100	0·0

The reason for the higher mortality obtained with 'sugar' bait is at present undetermined. There was not any visible difference in the quantity of bait eaten, although this is very difficult to estimate. There may be a difference in palatability between the 'sugar' and 'standard' baits without any corresponding difference in attractiveness. It is possible, although physiological evidence is lacking, that dextrose may have some influence on the permeability of the gut membranes to

metaldehyde. Dissection of poisoned slugs failed to show any obvious difference in the effect of the two types of bait on the gut.

Metaldehyde-bran baits compacted into 'biscuit' form

The disadvantages of metaldehyde-bran bait are that it rapidly goes mouldy under warm humid conditions and is readily washed away by rain, particularly when it is broadcast and not placed in protected heaps. Its use in experimental work for determining the relative populations of slugs on trial plots is hampered by the tendency of the bait to blow about in high wind, thus increasing the effective trapping area of the bait to an indeterminate degree. An additional drawback is that once the baits have been placed in position it is difficult to remove them so as to leave no trace of bait behind. Such fragments of the bait may easily influence subsequent trials on the same ground.

In order to overcome these difficulties a form of compacted bait was devised. In the first trials plaster of Paris was used as the binding agent. This was suggested by the observation of Barnes & Weil (1942) that flour and calcium carbonate set into a hard paste after rain, becoming proof against high wind, and by the present author's remembrance of feeding *Helix pomatia* L. on blocks of powdered lime and bran to induce heavy shell formation. The 'biscuits' were made from a mixture containing 1 vol. of dry plaster of Paris, 1 vol. of metaldehyde and 10 vol. of bran, the whole being thoroughly mixed. This mixture was then wetted sufficiently for it to be pressed into coherent lumps and moulded into biscuit form in a circle of tin 3 in. in diameter and $\frac{1}{4}$ in. deep. The tin mould was removed and the 'biscuit' sun-dried until it had set almost brick-hard.

The 'biscuits' were attractive to various species of slugs and snails and showed much greater resistance to mould than the 'standard' bait. They were easy to handle in experimental work, and when dried after use showed only small loss in efficiency. The catches on 11 consecutive nights on twenty 'standard' baits and twenty plaster of Paris 'biscuits' showed that the 'biscuits' caught slightly more *Milax gracilis* (Fig. 4) than did the 'standard' baits. The catch of *Arion hortensis* (Fig. 5) was almost the same on the two types of bait.

It was found, however, that the plaster of Paris 'biscuits' completely failed to kill any of the slugs trapped by them even under conditions when the 'standard' bait was causing a 90% mortality. The reason for this is not known; it is possible that the calcium ions derived from the plaster of Paris had an inhibiting effect on the absorption of metaldehyde by the gut lining (cp. the effect of dextrose on mortality). Dissection of slugs attracted to the 'biscuits' proved conclusively that an appreciable quantity of bait was eaten, and that the absence of fatal results was not due to the inability of the slugs to feed on the biscuits. It was therefore necessary to devise some form of bait which had the advantages of both plaster 'biscuit' and 'standard' bait. On the suggestion of Mr L. N. Staniland, Advisory Entomologist, Western Province, a waterproof casein glue was treated as the matrix of a 'biscuit' bait. One

part by volume of a commercial casein glue, 'Laitzo XXX', was mixed with its own volume of water, as recommended by the manufacturers,* and allowed to stand for

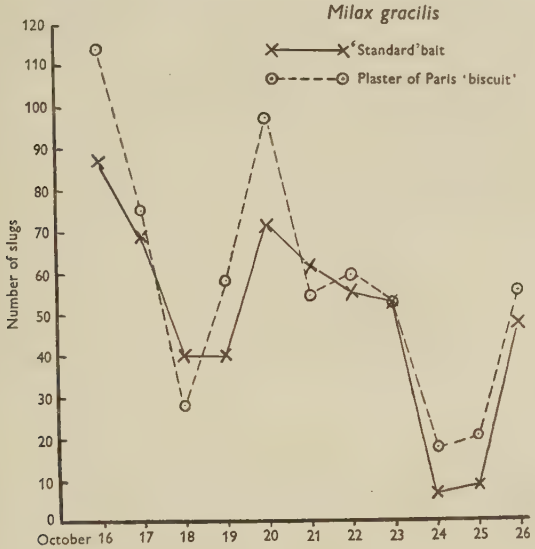

Fig. 4.

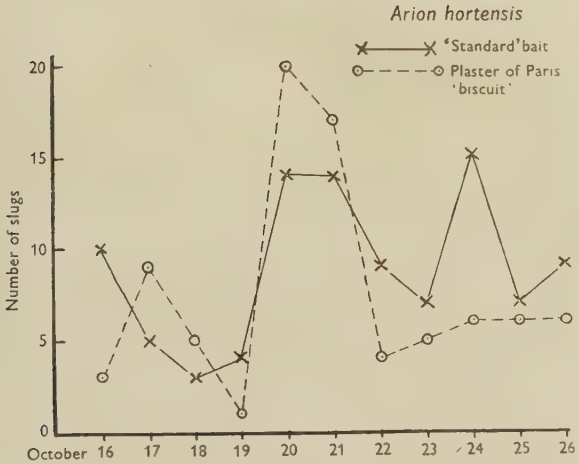

Fig. 5.

Figs. 4, 5. Comparison of the catch of slugs by 'standard' bait and by plaster of Paris 'biscuit'.

15 min. The mixture was then diluted by the addition of a further 3 vol. of water. The diluted glue was then mixed thoroughly with 20 vol. of bran (coarse millers'

* Casein Industries Ltd., Battersea, London.

offals) containing 1 vol. of metaldehyde. The resultant mash was moulded into biscuit form in exactly the same way as described for the plaster 'biscuits'. It was necessary to dust the board on which the 'biscuits' were made with a fine powder derived from the fragments of previously made casein 'biscuit' in order to prevent 'biscuits' from sticking to the board on drying. Batches of about fifty 'biscuits' at a time were made on a plywood board and dried under gas radiators. If the temperature of the air around the 'biscuits' rose above 40° C. during the drying process there was loss of metaldehyde, a fine 'growth' of needle-like crystals arising from the 'biscuits' by sublimation. After drying for approximately 12 hr., the 'biscuits' were turned over and dried for a further 6 hr. on the reverse. They were then quite hard but not as brittle as the plaster 'biscuits'. When the 'fine millers' offals' was used it was necessary to double the dilution of the glue, as the finer bran absorbed much more water than the coarse.

Under the conditions of hand manufacture it was not found possible to reduce the quantity of the glue, as not sufficient pressure could be used to cause the dryer mash to cohere.

TABLE 6. *The mortality of slugs caught by 'standard' baits and casein-metaldehyde-bran 'biscuits'. Twenty replicates of each type of bait. * indicates a significant difference from 'standard' bait*

	Milax gracilis		*Arion hortensis*		*Agriolimax reticulatus*	
	No. caught	% killed	No. caught	% killed	No. caught	% killed
'Standard' bait	446	56 ± 8·7	220	71 ± 7·7	358	65 ± 8·1
Casein 'biscuits'	556*	79 ± 5·6*	196	80 ± 4·4	460*	88 ± 6·5*

The 'biscuits' could be kept, apparently indefinitely, in an airtight tin without deterioration. They did, however, absorb moisture in a very humid atmosphere or from damp soil and become leathery, but showed very little tendency to go mouldy.

In field trials casein-metaldehyde-bran 'biscuits' were compared with 'standard' baits, both baits being covered with 6 in. squares of blackened glass. In this experiment, of which the summary appears in Table 6, twenty replicates of each bait were used. Significantly higher catches and kills of *Milax gracilis* and *Agriolimax reticulatus* were obtained with the 'biscuits' than with the 'standard' baits, but there appeared to be no difference between the baits with *Arion hortensis*. In a similar experiment the baits were left down for seventeen successive nights in the same positions, and the catch removed each morning. The catches of *Milax gracilis* (Fig. 6) and *Arion hortensis* (Fig. 7) confirmed the result of the previous experiment. The difference in response of the two species of slug to climatic conditions is strikingly shown in these figures. The submaximum of the numbers of *Milax gracilis* caught occurred on 12 February and the maximum on 18 February, corresponding to the two peaks of night minimum temperatures. *Arion hortensis* had the maximum and submaximum reversed, and a study of the rainfall figures shows that there was

ten times as much rain on 12 February as there was on 18 February. During the first 4 days of the experiment the low minimum temperature was a limiting factor in the activity of both species, and in spite of heavy rain the numbers of *A. hortensis* caught were very low.

At the end of the 17 nights' exposure the 'standard' bait had gone very mouldy, but most of the 'biscuits', although very damp and inclined to break up if not handled carefully, showed no sign of mould. Some loss of efficiency in attracting slugs was noticed with 'biscuits' that had been exposed for a number of nights

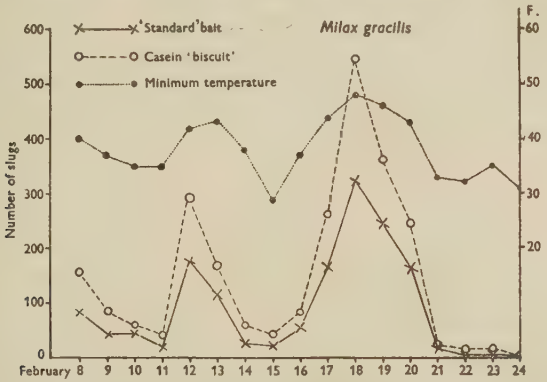

Fig. 6.

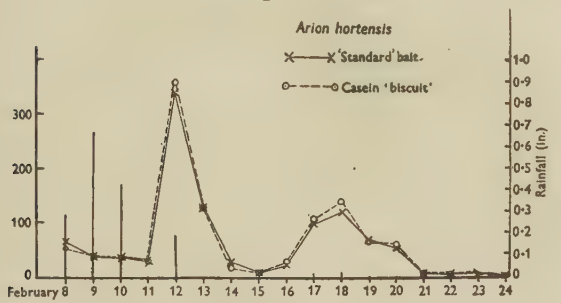

Fig. 7.

Figs. 6, 7. Comparison of the catch of slugs by 'standard' bait and by casein 'biscuits'.

consecutively. This conclusion is not to be drawn from the results in Figs. 6 and 7, as in this experiment the baits remained in the same position each night, and the area around each bait was gradually depleted of slugs. If the 'biscuits' were dried after use and the surfaces, particularly the edges, were scraped with a wire brush to remove the layer of slime and soil particles, the efficiency of the 'biscuits' was almost entirely restored.

An experiment laid out on a horticultural plot on Seale-Hayne College farm clearly demonstrated this renovation of the 'biscuits'. Twenty-four replicates of

four types of bait were used: 'standard', fresh 'biscuit', 'biscuits' after 21 nights' exposure and 'biscuits' renovated after similar exposure. Results (Table 7) show that while there was a significantly greater catch of *Milax gracilis* on the fresh 'biscuit' than on the old used 'biscuit', the catches on the renovated 'biscuit' were remarkably similar to those on the fresh 'biscuit'. This is of considerable importance to growers of market-garden crops, to whom the labour of picking up and renovating the 'biscuits' is within the bounds of economic possibility. In practice the 'biscuits' would not be left down for a period as long as 21 consecutive nights, and, given reasonable treatment, they might last for 12 months.

TABLE 7. *Numbers of* Milax gracilis *caught on twenty-five replicates each of 'standard' bait, fresh casein 'biscuit', old 'biscuit' and renovated 'biscuit'*

April 1945	'Standard' bait	Fresh 'biscuit'	Old 'biscuit'	Renovated 'biscuit'
4	51	87	54	78
5	124	183	89	166
6	85	80	41	102
7	130	130	71	122
8	23	46	21	52
9	34	66	28	52
10	7	23	13	26
Totals	454	615	317	598

The probable reason for the larger catch of *M. gracilis* on casein 'biscuits' is that it has a higher protein content than 'standard' bait. The protein content of casein 'biscuit' was calculated from a nitrogen determination by Kjeldahl analysis as 2·58%; that of the dried blood-metaldehyde-bran bait as used in the experiment described above as 4·44% and that of the 'standard' bait 1·91%.

M. gracilis has decided carnivorous habits and is readily attracted to bones to which fragments of meat are adhering. It also feeds on other slugs (Barnes & Weil, 1945), and Thomas (1947) observed cannibalism in this and related species in captivity. On one occasion *M. gagates* Drap. was found eating an immature *Testacella haliotidea* Drap. Slugs of the genus *Testacella* feed on worms and other slugs (Ellis, 1926), so this was a reversal of the usual habit. There was no evidence as to whether the *Milax* had attacked the *Testacella* alive or merely found it dead. It seems significant that *Arion hortensis* is not attracted to either casein 'biscuit' or dried-blood bait, more than to 'standard' bait, as this species lives almost entirely on the vascular tissues of plants and fungi.

ARSENICAL BAITS IN 'BISCUIT' FORM

Various forms of arsenical baits have been used against slugs and snails. Basinger (1923, 1931 and 1940) reported the successful use of a mash containing 1 part of calcium arsenate to 16 parts of bran against the snails *Helix* (*Euparypha*) *pisana* Müll. and *H. aspersa* in California. Lange & MacLeod (1941) suggested that a bait containing both calcium arsenate and metaldehyde was superior to baits containing

either poison separately. Thomas (1944) stated that Paris green and bran bait was inferior to metaldehyde and bran baits against *Agriolimax reticulatus*.

To test the efficiency of 'biscuits' containing an arsenical as well as metaldehyde, casein glue 'biscuits' were made containing 1 vol. of metaldehyde, 1 vol. of Paris green and 19 vol. of bran. A trial of these 'biscuits' which set satisfactorily was made in October 1945 at Seale-Hayne College, Newton Abbot, Devon. Twenty Paris green 'biscuits' were tested against twenty 'standard' casein 'biscuits' and twenty 'standard' baits. The catches and mortality of slugs (Table 8) in this experiment

TABLE 8. *Numbers of slugs caught and percentage killed by Paris green 'biscuits', 'standard biscuits' and 'standard' baits. * Indicates a significant difference from 'standard' bait, ** a significant difference from 'standard biscuit'*

		'Standard' baits	'Standard biscuits'	Paris green 'biscuits'
Milax gracilis:	No. caught	420±46	582±65*	320±25**
	% killed	67±13	79±11	70±15
Arion hortensis:	No. caught	86±25	42±12*	4**
	% killed	58±14	65±17	75

showed that Paris green apparently acts as a slight deterrent against *Milax gracilis* and is actively repellent to *Arion hortensis*. The killing power of the two types of 'biscuit' was not significantly different. It is possible that an arsenical containing a less irritant base than copper might be more effective, but the use of calcium arsenate may have the same disadvantage as the use of calcium sulphate in the plaster of Paris 'biscuits'.

The use of any arsenical in 'biscuit' form would be very dangerous, due to the risk of the 'biscuits' being eaten by children or domestic animals.

Broken metaldehyde-bran 'biscuit' as a broadcast bait

The durability and resistance to moulding of the casein 'biscuit' suggested that it might be used in small pieces as a broadcast bait. When broadcast, such a broken 'biscuit' bait is not readily washed away by rain nor dragged down into the soil by earthworms. The latter cause of loss was noticeable with ordinary metaldehyde-bran bait and more especially when wheat 'cavings' had been used instead of bran.

Broken 'biscuit' bait was made from 'standard' casein 'biscuits' broken by hand into approximately $\frac{1}{4}$ in. cubes. On breaking up the 'biscuit' great care was necessary to ensure that the glue was evenly mixed with the bait and was not lumpy; local high concentrations of glue in the 'biscuit' made it almost impossible to break by hand. The glue must be added to an equal volume of water very gradually and stirred continuously to prevent clotting.

When sufficient 'broken biscuit' had been prepared a plot of fallow horticultural land, which had carried potatoes in the previous year, was baited with 'standard' casein 'biscuits' in order to determine the distribution of slugs on it. The plot was a rectangle 20 × 28 yd. Forty 'standard' casein 'biscuits' were placed on each half of

the plot in four rows of ten 'biscuits' each. The catch was examined on the following morning, the 'biscuits' then being removed. Three species of slugs were present in sufficient numbers, and there was a statistically significant difference in their distribution on the two halves of the plot (Table 9). The half of the plot with the higher population of slugs was then broadcast by hand with 'broken biscuit' at the rate of 28 lb./acre. After 1 week, during which the weather was not particularly favourable for slug activity, both halves of the plot were trapped again, using 108 'standard' casein 'biscuits' on each half. This increase in the number of traps was considered necessary because the soil surface had become too dry for any considerable activity of slugs. The slugs caught on the two halves were counted the next morning, and the difference between the two halves was used as an index of the mortality of slugs on the treated half.

TABLE 9. *Trial of 'broken-biscuit' slug bait at Bishopsteignton, South Devon*

	No. of slugs caught prior to baiting 10 May 1945. Forty 'standard biscuits' per plot		No. of slugs caught 1 week after broadcasting with 'broken biscuit' 21 May 1945. 108 'standard biscuits' per plot		
	Experimental plot	Control plot	Experimental plot	Control plot	Apparent % kill
Milax gracilis	54 ± 9	44 ± 12	3	159	98
Arion hortensis	136 ± 42	108 ± 30	13	339	96
Agriolimax reticulatus	48 ± 4	53 ± 6	3	40	92

The results of this test (Table 9) show that there had been an apparent kill of over 90% of each of the three species. The difference in level of activity on the date of pre-trapping and the final trapping does not invalidate the strict comparison of the catches on each half-plot separately, provided that the total catch on the control plot was sufficiently great on both dates. Thus, if only ten slugs in the final trapping had been taken on the control plot and none on the broadcast plot, it would not have been valid to conclude that the mortality on the treated plot had been 100%.

A similar experiment on an adjoining piece of ground was attempted, but was spoilt by a stray domestic fowl which ate practically all the 'biscuit'. The bird did not appear to suffer any ill-effect as the result of eating at least 1 lb. of 'broken biscuit', and its egg-laying was not interrupted.

A further trial of 'broken biscuit' was made in October 1945 on a young strawberry bed at Seale-Hayne College. The plot, about $\frac{1}{10}$ acre, was baited with twenty-five 'standard' glass-covered 'biscuits' on the centre of each half, in a 5×5 1 yd. lattice arrangement. The weather conditions were extremely favourable for *Milax gracilis* but less so for *Arion hortensis*. Only these two species were present in sufficient numbers for statistical analysis. The numbers of *Milax gracilis* (Table 10) were not significantly different on the two halves of the plot, but *Arion hortensis*

TABLE 10. *Trial of 'broken-biscuit' slug bait at Seale-Hayne College, South Devon. Experimental plot broadcast on 22 October* 1945 *and both plots re-broadcast on 20 November* 1945

No. of slugs caught on twenty-five traps per plot

	Milax gracilis			*Arion hortensis*		
	Exp. plot	Control plot	% kill	Exp. plot	Control plot	% kill
21 Oct.	2211	2335	—	217	97	—
29 Oct.	24	392	94	9	18	78
6 Nov.	46	355	87	11	30	84

No. seen dead per 20 sq.ft.

	Milax gracilis			*Arion hortensis*		
	Exp. plot	Control plot	% kill	Exp. plot	Control plot	% kill
23 Oct.	82±30	0·0	—	10±4	0·0	—
24 Oct.	12±4	0·0	—	4±1	0·0	—
5 Nov.	32±9	0·0	—	4±2	0·0	—
21 Nov.	102±36	408–84	75	14±4	26±7	64

was approximately twice as abundant on the one half as on the other. The half with the higher population of *A. hortensis* was selected as the experimental plot and broadcast with 'broken biscuit' at the rate of 28 lb./acre on 22 October. The following morning a count was made of the numbers of freshly dead or moribund slugs in the central area of each plot. For this purpose a 1 ft. square wire frame was placed on the ground without the observer looking where it was put, in order to avoid prejudice in the exact placing of the frame. The slugs on the ground within this frame were then counted. Twenty such counts were made on each half-plot, the frame positions being in four rows of five, approximately 1 yd. apart. This count showed that there had been a kill of approximately 180,000 *Milax gracilis* and 26,000 *Arion hortensis* per acre on the treated plot. There were no dead slugs on the control plot. It is not possible to estimate the efficiency of the bait from these figures, but they are of interest in providing evidence of the numbers of slugs active on one night over the whole surface of a given area. Similar surface counts were made on 24 October and again on 5 November 1945. In these counts it was necessary to distinguish between slugs killed on the night previous to the count and those killed on preceding nights; therefore only newly dead and moribund slugs were counted. The total kill on the treated plot for the three nights was approximately 275,000 *Milax gracilis* and 50,000 *Arion hortensis* per acre.

One week after broadcasting the bait both plots were again trapped with twenty-five 'standard biscuits' in the central area of each plot. The baits were examined the following morning and the counts, when compared with the pre-baiting trial, showed that the kill of *Milax gracilis* and *Arion hortensis* had been 94 and 78%

respectively. It was necessary to make allowance for the difference in the original population level of *A. hortensis* on the two half-plots. A similar baiting was made on 6 November, and the kills were calculated as 87 and 84% respectively. This was considered to be a reasonable confirmation of the previous result.

It has been suggested that this method of estimation of percentage kill gives too high a figure because the 'biscuits' on the treated plot have to compete for slugs with the broadcast bait and the dead slugs on the surface, which are themselves attractive to healthy slugs. The 'biscuits' on the control plot have no such competition. A new technique was therefore devised whereby both the treated and control plots were subsequently broadcast with 'broken biscuit' at the rate of 56 lb./acre. This heavy dose was considered sufficient to 'swamp' any effect of the residue of the first broadcast bait. Only the central 100 sq.yd. of each plot was thus treated. On the morning following this second application of broadcast bait, surface counts on both plots were made with the wire frame as described above. The figures obtained by these counts (Table 10, bottom) gave kills of 75 and 64% for *Milax gracilis* and *Arion hortensis* respectively, thereby confirming the suggestion that the earlier estimates had been too high.

A comparison of the efficiency of the 'broken biscuit' with that of ordinary metaldehyde-bran bait broadcast was attempted on an acre of horticultural land at Coombe-in-Teignhead, Devon. The pre-broadcast baiting showed that there was a fairly even distribution of *A. intermedius* Norm., *A. circumscriptus* Johnst. and *Agriolimax reticulatus*, although the weather was not sufficiently favourable to obtain big catches of slugs. On 2 May 1946 one-third the area was broadcast with 'broken biscuit' and one-third with bait containing 1 vol. of metaldehyde to 30 vol. of bran; the remaining third was left untreated as a control. The weather subsequent to the broadcasting of the bait remained too dry for any considerable activity of slugs, and it was not until 10 June that it was thought advisable to assess the result. By this time no trace of the bran bait remained, but most of the larger pieces of the 'broken biscuits' were still recognizable. The results of the post-broadcast baiting, in which twenty 'standard biscuits' were used on each plot, showed (Table 11) that there had been a considerable hatch of *Arion intermedius* and some hatch of *A. circumscriptus* since the original baiting test. The numbers of *A. circumscriptus* and *Agriolimax reticulatus* were still very low, even on the control plot, and there were no significant differences in the kill of any of the three species.

A further attempt in October 1946 to compare the efficiency of these broadcast baits was made at the Horticultural Department, Seale-Hayne College, where the population of slugs was considerably higher than at Coombe-in-Teignhead. The plots, bare fallow following onions, were 14 yd. wide by 38 yd. long. The pre-broadcast trapping showed (Table 12) that the plot afterwards selected as the control had significantly less *Milax gracilis* than the plot chosen for the metaldehyde-bran bait, but was not significantly different from the 'broken-biscuit' plot. After broadcasting with the two baits, both at the rate of 28 lb./acre, on 18 October,

surface counts were made on each plot on 19, 22 and 23 October. It was found that the kill on the 'broken-biscuit' plot persisted at a higher level than that on the

TABLE 11. *Trial of 'broken-biscuit' slug bait at Coombe-in-Teignhead, South Devon. Experimental plots broadcast 2 May* 1946

	No. of slugs caught prior to baiting 10 April 1946. Twenty traps per plot		
	Metaldehyde-bran plot	'Biscuit' plot	Control plot
Arion intermedius	29	36	25
Agriolimax reticulatus	24	32	39
Arion circumscriptus	23	29	19
	No. of slugs caught 5 weeks after baiting 10 June 1946. Twenty traps per plot		
	Metaldehyde-bran plot	'Biscuit' plot	Control plot
Arion intermedius	52	29	215
Agriolimax reticulatus	3	4	26
Arion circumscriptus	4	8	42
	Apparent % kill. 10 June 1946		
	Metaldehyde-bran plot	'Biscuit' plot	Control plot
Arion intermedius	79	90·6	—
Agriolimax reticulatus	88	84	—
Arion circumscriptus	90	81	—

TABLE 12. *Trial of 'broken-biscuit' slug bait at Seale-Hayne College experimental plots broadcast on* 18 *October* 1946

	No. of *Milax gracilis* caught on twenty traps per plot		
	Metaldehyde-bran plot	'Biscuit' plot	Control plot
Before baiting 16 Oct.	232 ± 58	177 ± 54	148 ± 24
After baiting 6 Nov.	597 ± 45	166 ± 18	749 ± 42
Apparent % kill	22·7	77·8	—
	Mean no. of *Milax gracilis* per sq.ft. on surface of plots after broadcasting bait		
	Metaldehyde-bran plot	'Biscuit' plot	Control plot
19 Oct.	3·6 ± 0·5	5·8 ± 0·7	0·7 ± 0·1
22 Oct.	0·6	2·8 ± 0·3	0·3
23 Oct.	0·3	2·7 ± 0·3	0·1

metaldehyde-bran plot. Thus, on the sixth night after broadcasting the bait, the number of *Milax gracilis* killed on the 'broken-biscuit' plot was approximately 118,000 per acre, while that on the metaldehyde-bran plot was only 13,000 per acre.

Heavy rain had followed the broadcasting and very little metaldehyde-bran bait was visible; the 'broken biscuit', although soft, had not been dispersed. The small number of slugs found on the control plot appeared to be due to the drift of some of the light metaldehyde-bran bait by wind as it was being broadcast.

The results of the post-broadcasting carried out on 6 November gave apparent kills of 23% on the metaldehyde-bran plot and 78% on the 'broken-biscuit' plot —a highly significant difference. Cultural operations prevented the experiment being concluded by rebaiting all the plots with 'broken biscuit' at 56 lb./acre and making surface counts, but the results of the previous experiment with this technique suggest that it would not have greatly altered the relative significance of the mortality figures for the two baits.

General conclusions and recommendations

The successful control of slugs by metaldehyde is dependent on the use of a bait containing not much less than 1 part of metaldehyde to 30 parts of bran and, when feeding-stuffs supplies permit, the addition of 1 part of crude sugar or molasses. On land infested with slugs of the genus *Milax*, which are the major pests of potatoes on horticultural land (Wilson, 1943), the addition of protein in some form such as dried blood is advisable.

The climatic conditions requisite for optimum results are warm, damp nights, favouring a high percentage activity of slugs, followed by drying conditions during the daytime. Heavy rain at night is found to inhibit the activity of many slugs and is also detrimental to the bait. Where *Arion hortensis* is the most troublesome species a warm night with the probability of some rain should be selected for baiting. If, however, rain persists throughout the following day only a small kill of slugs can be expected.

Where root crops, potatoes and celery, whose subterranean parts are particularly attractive to slugs, are grown it is essential to clear the land of slugs before the crop is established, as no baits are yet known which are sufficiently attractive to bring the slugs up from an ample supply of favoured food. In this connexion it is also necessary to bait as large an area as possible simultaneously, otherwise the crop will be attacked by slugs migrating from the unbaited area around it. Where possible, baiting for slugs should be carried out on bare ground offering little cover to the partially poisoned slugs.

The commercial use of casein glue-metaldehyde-bran bait is dependent on its being manufactured on a large scale, as the bait cannot easily be made in sufficient quantity by individual growers. The cost of manufacturing this bait wholesale is difficult to estimate, since modifications in the manufacture, such as greater pressure on the mash, may enable reductions to be made in the quantity or quality of glue used. The present cost of materials for broadcasting an acre at 28 lb./acre is 17*s*. Labour costs under the conditions of experimental manufacture would be prohibitive, but should be very considerably reduced in wholesale manufacture.

The superiority of 'broken biscuit' over any other forms of bait, due to its durability, make it desirable that its production on a commercial scale should enable growers, especially growers of valuable horticultural crops, to use it extensively.

Grateful acknowledgements are due to Mr L. N. Staniland, Advisory Entomologist in the Western Province, for his ever-ready assistance and helpful suggestions, and to all his staff in the Department of Plant Pathology, Seale-Hayne Agricultural College, Newton Abbot, Devon, for their help in making the counts and preparing the baits. We are also indebted to Mr O. D. Kimble for his kindness in allowing the use of the horticultural ground at Seale-Hayne College for experimental work, and to Mrs P. V. Luxton and Mr Lee for allowing similar facilities on their land at Bishopsteignton and Coombe-in-Teignhead respectively. Dr H. F. Barnes and Mr J. W. Weil of Rothamsted Experimental Station have been most valued critics throughout the whole course of the work, and they have given much useful advice on the interpretation of the results.

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(*Received* 8 *August* 1947)

THE FORMATION OF INSECTICIDAL FILMS ON BUILDING MATERIALS

III. SUPPLEMENTARY LABORATORY TESTS ON SIZE AND GELATIN AS PRETREATMENTS

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The success of size and gelatin as pretreatments was not confined to films of pyrethrins in oil. Pretreatment of cement with 10% w/v size and 5% w/v gelatin greatly prolonged the toxic life of films formed by other insecticides in oil solution.

The effects of adding different substances to size and gelatin solutions as pretreatments were investigated. 5% w/v size or gelatin solutions containing suspended lime or distemper powder were, with the exception of gelatin containing distemper, less effective pretreatments than size or gelatin solutions alone. Magnesium silicofluoride, benzoic acid and salicylic acid, at concentrations up to 0·5% w/v, appear suitable as preservatives for 5% w/v gelatin. These three substances are, however, unsuitable for inclusion in size solutions, as they cause precipitates to form. Glycerin or turkey-red oil at concentrations of 0·5% v/v, appear suitable as plasticizers for inclusion in both 5% w/v size and 5% w/v gelatin solutions.

Suitable pretreatment of building materials can greatly prolong the toxic life of insecticidal films formed upon them (Parkin & Hewlett, 1946). Hewlett & Parkin (1947) described tests which led them to select size and gelatin for more detailed investigation as pretreatments. Further laboratory tests were carried out on these two substances, and are now described.

Experimental technique

The free surface of cement was the substrate used throughout the experiments described here. Those samples pretreated were painted with 10 or 5% w/v aqueous solutions of size, or 5% w/v aqueous solutions of an edible grade of gelatin. The test insects were flour beetles, *Tribolium castaneum* Herbst, bred and handled as described by Parkin & Green (1943). They were 3–5 or 4–6 weeks old, according to the particular experiment, when first confined on the films. The method for preparing the cement samples, and the techniques of pretreatment, spraying and exposure of the insects on the films, were the same as before (Parkin & Hewlett, 1946; Hewlett & Parkin, 1947).

On the basis of experiments on the decrease of toxicity of films with increasing age on pretreated surfaces (Hewlett & Parkin, 1947), the beetles were confined on films 24 hr. and 7 days old, to provide some information on the rate of loss of toxicity. On pretreated surfaces many of the films 24 hr. old were so toxic as to kill all the beetles confined on them, whereas some of the similar films 7 days old were

not, so that sometimes the toxicities of the older films could be compared when those of the younger could not.

The toxicity of films formed by different insecticides on pretreated cement

Hewlett & Parkin (1947) had selected size and gelatin for further work by means of tests with pyrethrum only, so it was clearly desirable to discover whether pretreatment with size and gelatin would increase the persistence of films formed by other insecticides.

Cement samples, pretreated and not pretreated, were sprayed with different insecticides, and beetles confined on the films. The insecticides were:

(*a*) 1·6% w/v pyrethrins in Shell Oil P31.

(*b*) 1·0 and 0·5% w/v crude D.D.T. in P31. The crude D.D.T. contained 74·6% of the *p*, *p*′-compound.

(*c*) 20 or 10% v/v in P31 of a concentrate (A) containing 50% by volume of a petroleum distillate and 50% of a mixture of β-butoxy-β′-thiocyanodiethyl-ether and β-thiocyano-esters of certain higher fatty acids.

(*d*) 20 or 10% v/v in P31 of a concentrate (B) containing bornyl and isobornyl thiocyano-acetates. The concentrates A and B were of commercial origin.

The results are given in Table 1. The mortality in the sprayed and unsprayed controls was 0–16%.

Table 1. *The toxicity to* Tribolium castaneum *of films formed by* 1·44–1·56 mg./sq.cm. *of various insecticides in P31 on pretreated cement: exposure period* 6 *days*

0	0– 20%
+	21– 95%
++	96–100%

} mortality in batches of 50 insects

	Pretreatment			
	10% w/v size		5% w/v gelatin	
Insecticide	24 hr.*	7 days*	24 hr.*	7 days*
1·6% w/v pyrethrins	++	++	++	++
1·0% w/v D.D.T.	++	++	++	++
0·5% w/v D.D.T.	0	0	++	+
20% v/v A	+	0	++	++
10% v/v A	++	0	++	0
20% v/v B	0	0	++	0
10% v/v B	0	0	0	0

* Age of film when insects put on.

Table 1 shows that pretreatment with 10% size or 5% gelatin increased the persistence of most of the insecticidal films. This was not unexpected, since all the insecticides were oil-based, and size and gelatin coatings, being hydrophilic, are relatively impermeable to oil. 5% gelatin was evidently superior to 10% size in making the films persistent.

In general the films formed by 1·6% pyrethrins and 1·0% D.D.T. were more toxic than the others. In tests by the film technique of Parkin & Green (1943) 1·0% D.D.T. was somewhat more toxic to *T. castaneum*, and 0·5% somewhat less so, than 1·6% pyrethrins. Equivalent concentrations of pyrethrins and of the concentrates A and B are not known, so that, where films of (*c*) and (*d*) killed less than 20% of the insects, it is at present impossible to say whether the pretreatment was unsuited to (*c*) or (*d*), or whether the intrinsic toxicity of these preparations was low.

Up to this point in the investigation size and gelatin had been used at concentrations of 10 and 5% respectively. Tests showed that pyrethrum films on cement pretreated with 5% solutions of either substance were highly toxic; when insects were exposed on films 24 hr. and 7 days old all the insects were killed after 6 days' exposure. In view of these results 5% w/v size, and not 10%, was used in later experiments, together with 5% w/v gelatin.

The effect of adding various substances to size and gelatin

(1) *Fillers*

If a suspension of lime or distemper in size or gelatin was an effective pretreatment, it might be possible to pretreat and limewash or distemper a surface in one operation. To investigate this possibility, limewashes and distempers were prepared by adding lime or distemper powder to 5% w/v size and 5% w/v gelatin. Enough powder was added to each solution to form an opaque, pigmented coating when the suspension was applied as a single coat to cement samples and allowed to dry. Table 2 gives the results of tests of the toxicity of films of 1·6% pyrethrins deposited on such pretreated samples.

Table 2. *The toxicity to* Tribolium castaneum *of films formed by* 1·48–1·57 mg./sq.cm. *of* 1·6% *w/v pyrethrins in P*31 *on cement pretreated with size and gelatin containing lime and distemper fillers; exposure period* 6 *days*

Pretreatment	Mortality (%)	
	24 hr.*	7 days*
None	6	—
5% w/v size	100	100
5% w/v size + lime	6	2
5% w/v size + distemper	0	0
5% w/v gelatin	100	100
5% w/v gelatin + lime	98	2
5% w/v gelatin + distemper	100	98

* Age of film when insects put on. The figure for the 24 hr. initial film age is the percentage mortality from one batch of fifty insects, that for 7 days initial film age is the mean from two such batches.

From Table 2 it is evident that inclusion of a filler in size or gelatin can greatly reduce the persistence of the insecticidal film. Films formed on lime and distemper in size had lost their toxicity completely after 24 hr. Those on lime in gelatin were

toxic after 24 hr., but non-toxic after 7 days, while those on distemper in gelatin remained highly toxic for 7 days at least. Thus, the inclusion of the fillers reduced the film-supporting capacity of gelatin less than that of size, and the film-supporting capacity of gelatin was reduced much more by lime than by distemper. Possibly the latter effect was connected with the greater particle size of the lime.

In general, therefore, a filler is likely to reduce the effectiveness of size and gelatin as pretreatments, but a suspension of distemper in gelatin might be satisfactory.

(2) *Preservatives*

Under exceptionally humid conditions moulds and bacteria might attack coatings of size and gelatin. Magnesium silicofluoride, benzoic acid and salicylic acid were therefore investigated with a view to their use as preservatives in size and gelatin. Galloway (1937) recommended that the last two substances be used in water paints at a concentration of 0·05% w/v. However, the inclusion of 0·05% of any of the three substances in 5% w/v gelatin solutions did not prevent mould and bacterial growth in less than a week when the gelled solutions were kept in closed flasks at about 20° C.—admittedly most favourable conditions for mould and bacterial growth. On raising the concentration of preservative to 0·5%, 5% gelatin solutions could be preserved under similar conditions for 1 month, and the gelled solution containing benzoic acid did not liquefy until it had been stored for 3 months.

5% gelatin solutions containing 0·5% of preservatives were applied to cement samples, and the pretreated surfaces compared for film-supporting properties, with surfaces pretreated with 5% gelatin alone. The surfaces were sprayed with about 1·5 mg./sq.cm. of 1·6% w/v pyrethrins, and the sprayed samples left for 24 hr. and 7 days respectively before insects were confined on them. After 6 days' exposure on the films all the beetles were dead on every sample, so that no diminution in film toxicity due to preservatives in the pretreatment was detected.

When the same preservatives were added to 5% w/v size at 0·5 or 0·05%, a white, flocculent precipitate rapidly settled out. Similar precipitation occurred on adding benzoic acid at 0·005%. The question of a preservative for size was pursued no further.

Thus, up to 0·5% of magnesium silicofluoride, benzoic acid, or salicylic acid could be added to 5% w/v gelatin without detracting seriously from the desirable properties of the gelatin. Possibly benzoic acid is the best preservative of the three. The same three preservatives are, however, unsuitable for addition to size.

(3) *Plasticizers*

Under dry conditions a coating of size or gelatin alone might contract sufficiently to remove itself, together with flakes of limewash if present, from the surface of the underlying building material. A suitable plasticizer, e.g. glycerin, turkey-red oil, or glucose syrup, in the size or gelatin would probably prevent this contraction.

Cement samples were pretreated with 5% w/v solutions of size and gelatin

containing 0·5% v/v glycerin or turkey-red oil, and the pretreated surfaces compared for insecticidal film-supporting efficiency with surfaces pretreated with similar solutions of size and gelatin alone. The technique was similar to that employed for testing gelatin containing preservatives. No reduction in film-supporting efficiency, due to the presence of the glycerin or turkey-red oil, was detected, and so in this respect these substances appear suitable as plasticizers for pretreatment coatings of size and gelatin.

Discussion

From experiments on the toxicity of films to insects, 5% gelatin appears to be a better pretreatment than 5% size. Table 2 indicates that 5% gelatin is superior to 10% size in making films of different insecticides more persistent; other tests (unpublished) suggested that, within limits, the higher the concentration of size in the pretreatment, the more persistent the film formed upon it, so that in this respect 5% gelatin should be more superior to 5% than to 10% size. Adding fillers affected 5% gelatin less adversely than 5% size. Finally, films formed by 0·8 or 0·4% w/v pyrethrins were more persistent on cement pretreated with 5% gelatin than on cement pretreated with 5% size (unpublished). Size, however, is less expensive than gelatin, and might therefore be preferable for practical use.

The next stage in the investigation was to attempt to discover whether size and gelatin would be effective pretreatments in practice; how they could be applied conveniently in practice; at what concentrations they should be applied; and whether preservatives or plasticizers were necessary. These trials will be described in the next paper of this series.

The work has formed part of the programme of research of the Pest Infestation Laboratory, and this account is published with the permission of the Department of Scientific and Industrial Research.

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(*Received* 2 *October* 1947)

D.D.T. IMPREGNATION OF SACKS FOR THE PROTECTION OF STORED CEREALS AGAINST INSECT INFESTATION

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(With Plate 3 and 1 Text-figure)

Cotton flour sacks, jute flour sacks, jute bran sacks and jute grain sacks have been impregnated with 1 and 5 % by weight of D.D.T. Treated and untreated sacks, each containing 1 cwt. of a mixture of grain (5 parts) and weatings (1 part), were exposed to severe infestation by adults of *Calandra granaria, Tribolium castaneum, Ptinus tectus, Oryzaephilus surinamensis* and *Ephestia elutella*. After storage for 15 weeks at a mean temperature of 21·3° C. and a mean relative humidity of 65 %, the numbers of adult insects in each sack were determined by sieving the contents.

The results with *Ephestia elutella* were inconclusive. The results with the four species of beetles showed that the eventual degree of infestation of the contents of the sacks was dependent upon the closeness of weave and the D.D.T. content of the sacking material. Impregnation of sacking with 1 % of D.D.T. should afford adequate protection against insect infestation to clean bagged goods stored in stowages which are not heavily infested. Impregnation with 5 % of D.D.T. should offer a very high degree of protection at all times and can almost entirely prevent infestation, if the treated material is of sufficiently close weave to afford some mechanical hindrance to penetration by insects.

Introduction

It is considered inadvisable at present to apply D.D.T. directly to stored foodstuffs for the prevention and control of insect infestation. More data must first be collected on the toxicity of this insecticide to man, especially when it is ingested in small quantities over very long periods. Sacks impregnated with D.D.T., however, would seem likely to contaminate little, if at all, the commodities they contain. Nevertheless, this point will require investigation before recommendations are made for the use of such sacks in practice, particularly for the storage of materials with a high fat content. In anticipation of the possibility that sacks impregnated with D.D.T. may eventually be declared safe for commercial use, experiments have been started to determine the value of D.D.T. impregnation of sacks for the prevention and control of insect infestation in grain and grain products. The experiments have also given some information upon the protection against insect infestation afforded by different types of untreated sacking.

The main part of the investigation involved the exposure of forty-eight sacks, each containing 1 cwt. (approx. 50 kg.) of suitable foodstuffs, to severe infestation from an externally applied, mixed population of four species of beetles and one species of moth, all commonly found in grain and flour. The sacks were kept in a close pile, one sack deep, on the floor of a room maintained at an approximately constant temperature and relative humidity. The insects were thus free to move from sack to sack. Observations were made upon the susceptibility of the different

species to D.D.T. impregnated on the different types of sacking and upon the ability of the insects to penetrate from the exterior to the interior of the bags.

EXPERIMENTAL MATERIALS

(1) *Sacking*

Four types of sacks were chosen as representative of the types in common commercial use. The following descriptions are preceded by a reference letter which is subsequently used, for brevity, in most of the tables of results. The relative size and weave of the threads are shown in Pl. 3, fig. 1. The materials from which the sacks were made were checked by the Plant and Animal Products Division, Imperial Institute.

A. *Cotton flour sacks.* White; closely woven twill; size, flat, 3 ft. 3 in. × 2 ft.; capacity 140 lb. flour.

B. *Jute flour sacks.* Grey-brown; fairly closely woven hessian; size, flat, 3 ft. 2 in. × 2 ft.; capacity 140 lb. flour.

C. *Jute bran sacks.* Yellow-brown; very loosely woven hessian; size, flat, 4 ft. 6 in. × 2 ft. 3 in.; capacity 112–140 lb. bran.

D. *Jute grain sacks.* Grey-brown; fairly closely woven twill, but of much coarser thread than the jute flour sacks; size, flat, 3 ft. 6 in. × 2 ft. 3 in.; capacity 180 lb. grain.

Sixty sound sacks of each type were provided by the Infestation Division,* Ministry of Food, who cleaned the sacks and fumigated them with HCN before delivery. Ten of each kind of sack were kept under insect-free conditions at the Pest Infestation Laboratory as untreated controls. The remainder were sent to the Chemical Defence Experimental Station, Ministry of Supply, for impregnation with D.D.T.; twenty of each type were returned impregnated with 1% w/w D.D.T., and a further twenty with 5% w/w D.D.T.

According to C.D.E.S. the impregnations with 1% D.D.T. were carried out from a bath of 4·2% D.D.T. in white spirit by the manufacturing method used for D.D.T.-impregnated shirts. Loads, each of 20 lb., were soaked in 20 gal. of solution for 5 min., hydro-extracted and dried. In order to obtain the requisite impregnation, a pick-up of some 24% of solution was required, so that the periods of centrifuging were brief (30–90 sec.) and might cause some difficulty in practice. Drying took approximately 1 hr. at 130–140° F. The impregnations with 5% D.D.T. were carried out by the standard laundry process, using A.T.S.O. no. 2 emulsion. The sacks were given a standard shirt wash (1 lb./1 gal.) at 100° F. (breakdown, two washes, three rinses), the A.T.S.O. being added in the third rinse. After hydro-extraction the sacks required some 2 hr. for drying at 130–140° F. An excess of A.T.S.O. had to be used, because exhaustion was by no means complete; one-third of the chemical was wasted with jute flour and jute bran sacks, and one-sixth with cotton flour and jute grain sacks. Chemical determinations by C.D.E.S. of the D.D.T. contents of samples of the various types of sacks yielded the data shown in Table 1.

When the sacks were received at the Pest Infestation Laboratory after impregna-

* Now of Ministry of Agriculture and Fisheries.

tion, those containing 1% D.D.T. showed no change in appearance, but smelt slightly, though definitely, of D.D.T. Those containing 5% D.D.T. were all rougher in appearance than the corresponding untreated sacks, were somewhat shrunken, and smelt strongly of D.D.T.

TABLE 1. *Quantities of D.D.T. present on samples of impregnated sacks*

Type of sack	D.D.T. (%) based on air-dry weight of sacks: Samples	Mean
Impregnated with approximately 1% of D.D.T.		
Cotton flour	1·17, 1·15, 1·10	1·14
Jute flour	1·00, 0·99, 0·88	0·96
Jute bran	1·05, 0·95, 0·92, 0·88	0·95
Jute grain	1·17, 1·11, 1·07, 1·05	1·10
Impregnated with approximately 5% of D.D.T.		
Cotton flour	5·2, 4·6, 4·2, 4·2	4·5
Jute flour	4·6, 4·4, 4·4	4·5
Jute bran	4·4, 4·4, 4·4	4·4
Jute grain	4·8, 4·6, 4·6	4·7

(2) *Insects*

The four species of beetles used were *Calandra granaria* L., *Tribolium castaneum* Herbst, *Ptinus tectus* Boield., and *Oryzaephilus surinamensis* L.; the species of moth was *Ephestia elutella* Hb. Special arrangements were made to breed about 12,000 beetles of each species and about 3000 moths, so as to have them available at the anticipated time of starting the exposure of the sacks. All insects were bred at 25° C. and 70% R.H. When the various experiments were started, the majority of the beetles were 2–4 weeks old, and the moths 0–1 week old.

(3) *Food*

The sacks were filled with a mixture of English wheat (5 parts by weight) and fine weatings (1 part by weight). The weatings were added because the wheat alone would probably have contained too few broken grains to support a heavy infestation by the secondary pests (*Tribolium*, *Ptinus*, *Oryzaephilus*).

Provision of the grain and fine weatings was arranged by the Infestation Division, Ministry of Food. On receipt at the Pest Infestation Laboratory, both types of foodstuffs were separately fumigated with a mixture of ethylene oxide and carbon dioxide, to eliminate any active insect infestation. It was thought advisable to employ this safety measure, although the foodstuffs appeared uninfected. After thorough airing in the fumigation chamber, the foodstuffs were mixed by turning over piles of 6–7 cwt. with shovels; 1 cwt. (50 kg.) of the mixture was loaded into each sack.

EXPERIMENTAL METHOD

Four sacks of each combination of material (A, B, C or D) and treatment (0, 1 or 5% D.D.T.) were charged with food, and the open end of each sack was then doubled over, crimped and tied tightly to prevent entry of insects. A sack of each

type, after tying and labelling, is shown in Pl. 3, fig. 2. The forty-eight sacks were arranged on the painted concrete floor of a room, maintained at an approximately constant temperature and relative humidity, in a square pattern of seven sacks along each side with an empty space in the centre, as shown in Text-fig. 1. The letters A–D refer to the type of sacking; the figures 0, 1 or 5 between the strokes to the percentage of D.D.T.; and the final figures 1–4 to the replicates.

S.E.

	D/0/4	C/5/4	A/1/3	D/0/3	C/5/3	A/1/4	A/0/4
	D/1/4	B/0/2	C/5/2	D/5/1	B/1/2	C/0/2	D/5/4
	B/5/3	A/1/2	B/1/1	A/0/1	C/1/1	D/5/2	B/1/3
N.E.	C/0/3	C/5/1	D/0/1		B/0/1	A/5/1	A/0/3	S.W.
	D/1/3	B/5/2	A/1/1	C/0/1	D/1/1	C/1/2	D/5/3
	B/5/4	A/0/2	D/1/2	B/5/1	A/5/2	D/0/2	B/1/4
	C/0/4	C/1/4	A/5/3	B/0/3	C/1/3	A/5/4	B/0/4

N.W.

Text-fig. 1. Plan of positions of D.D.T.-impregnated sacks on floor.

The sacks were piled in loose contact with one another, and their arrangement was such that each impregnated sack was in contact in its respective row, column or diagonals, with two untreated controls. No particular merit is claimed for this disposition of the sacks, but it did offer the insects reasonable opportunities to migrate from treated to untreated sacks. There was a free passage of at least 2 ft. on all sides of the square, and insects could wander away from the group of sacks towards the walls. Windows were present in the north-east and south-west sides of the room.

The sacks were filled and put in position on 8–9 August 1946. On 9 August, when the lay-out was complete, 9600 each of *Calandra granaria*, *Tribolium castaneum*, and *Ptinus tectus* beetles were scattered as evenly as possible over the tops of the sacks. On 10 August 9600 *Oryzaephilus surinamensis* beetles were distributed. In addition, about 100 *Ephestia elutella* moths were liberated and thirty rolls of corrugated paper containing pupae were scattered on the tops of the sacks; the number of moths which eventually emerged was estimated at 2000–3000.

Two trays, about 3 ft. 6 in. × 1 ft. 9 in. and 1 in. deep, were placed on the floor of the room and kept filled with distilled water to help to maintain a satisfactory

level of relative humidity. Heat could be supplied to the room by hot-water radiators, the output of which could be partially controlled. Sudden or extreme fluctuations in atmospheric conditions were thereby avoided. Continuous records of temperature and relative humidity were obtained with a thermohygrograph which was frequently checked by a sling psychrometer. Mean temperatures and relative humidities were computed from the record charts with the aid of a planimeter.

Results

(1) *Intermediate examinations*

A superficial examination was made on 13 August, 4 days after the start. During this exposure period the mean temperature and relative humidity were 24·8° C. and 65% respectively. Considerable numbers of beetles had wandered from the sacks to the walls of the room and there died in a film of oil painted on the skirting to restrict their migration. The number of beetles effective as a source of infestation to the sacks was therefore much lower than the number of beetles originally introduced into the room. Many dead beetles of all four species were observed on the floor and more were found round the treated sacks than round the untreated. Some 80–90% of the beetles visible on the treated sacks appeared dead, contrasting with 20–30% on the controls. It was already apparent that *Ptinus* was the most resistant of the four species of beetles to the D.D.T.-impregnated sacking. By this time a large number of *Ephestia* moths had emerged. Many were resting on the walls of the room, but a considerable number were fairly uniformly scattered over all the sacks, irrespective of type or treatment.

On 28–29 August, 19 days after the start (mean temp. 24·8° C.; R.H. 67·4%), a count was made of the living and dead beetles in the folds on the top of each bag, but without untying the sack. Examination of the detailed results disclosed no differences which could be ascribed to the position of bags in the group, so the data within each set of four replicates have been totalled and are shown in Table 2.

Table 2. *Total numbers of beetles in top folds of sacks for each set of four replicates, 19 days after the start of exposure*

Sack type and treatment	Living beetles				Dead beetles			
	Cal.	*Trib.*	*Ptin.*	*Oryz.*	*Cal.*	*Trib.*	*Ptin.*	*Oryz.*
A/0	0	7	35	3	3	1	21	6
A/1	0	0	48	2	52	53	68	51
A/5	0	0	64	5	10	59	124	64
B/0	0	2	20	3	1	1	18	4
B/1	0	2	45	2	23	54	47	52
B/5	0	0	58	1	16	65	92	61
C/0	10	7	17	3	10	2	14	1
C/1	0	0	26	5	56	28	84	5
C/5	0	0	51	1	73	16	99	14
D/0	0	0	26	3	12	0	26	0
D/1	0	2	43	1	43	150	68	63
D/5	0	0	42	0	68	98	93	33

Unexpectedly small numbers of living *Calandra*, *Tribolium and Oryzaephilus* beetles were observed. *Ptinus* was more numerous, and more living beetles were found on the treated sacks than on the corresponding controls. The numbers of dead insects of all four species were much larger on the treated than on the untreated sacks of the same type. No count was made of *Ephestia* moths, almost all of which were dead and had fallen to the floor.

On 19 September, 7 weeks from the start (mean temp. 22·8° C.; R.H. 68%) samples of about 850 g. of foodstuffs were withdrawn from the top 20 cm. of the outer layer of replicates 3 and 4 of each combination of sacking and treatment. The samples were examined, by sieving, for the presence of insects. *Oryzaephilus* larvae, if present, were not distinguished from those of *Tribolium*, and no attempt was made to determine whether *Calandra* larvae were present within the grains. The results may be summarized as follows (insects alive, unless otherwise stated):

A. *Cotton flour sacks.* No adult insects were found. A few *Ptinus* larvae were seen in one untreated sack, but no infestation was observed in the contents of D.D.T.-treated sacks.

B. *Jute flour sacks.* One *Tribolium* beetle and moderate numbers of *Tribolium*, *Ptinus* and *Ephestia* larvae were observed in the controls. A few *Tribolium* larvae only were found in the 1% and no insects in the 5% D.D.T. sacks.

C. *Jute bran sacks.* Many *Tribolium* beetles were seen in the controls as well as *Tribolium*, *Ptinus* and *Ephestia* larvae. A few *Tribolium* beetles penetrated the treated sacks. *Tribolium* and *Ptinus* larvae were present in those impregnated with 1% D.D.T., but apparently absent from those with 5% D.D.T.

D. *Jute grain sacks.* Some *Tribolium* beetles had penetrated the untreated sacks; one dead *Tribolium* was seen in the samples from the 1% D.D.T. sacks, and one dead *Calandra* and two dead *Ptinus* in one of the 5% D.D.T. sacks. *Tribolium* and *Ptinus* larvae were observed in the controls, very few *Tribolium* larvae in the 1%, and no larvae in the 5% D.D.T. sacks.

(2) *Final examination*

A few living adult *Calandra*, *Tribolium*, *Ptinus* and *Ephestia* were observed on the sacks on 1 November, indicating that emergence of the F_1 generation had started. On 21 November, 15 weeks after the sacks had originally been exposed to infestation, examination of the contents was started; this examination was completed in about 10 days. The mixed grain and weatings from a sack were run relatively slowly through a sieving machine fitted with reciprocating 10-, 20- and 30-mesh screens. This separated the mixture into four fractions, and careful preliminary examination showed that adult insects were present only in that fraction retained on the 20-mesh screen. The numbers of living and dead beetles of each species in each sack were then counted. The location of sacks within the group on the floor seemed to have no influence upon the variations in the results among replicates, and the data have therefore been summarized in Table 3 to show the total numbers of insects per set of four replicates. *Ephestia* moths, pupae and larvae could not be satisfactorily

detected during the sieving and counting, and the scanty data for this species have been omitted from the table. During the whole exposure period the mean temperature was 21·3° C. and the relative humidity 65%.

TABLE 3. *Total numbers of beetles in contents of sacks for each set of four replicates, 15 weeks after start of exposure*

Sack type and treatment	Living beetles				Dead beetles			
	Cal.	*Trib.*	*Ptin.*	*Oryz.*	*Cal.*	*Trib.*	*Ptin.*	*Oryz.*
A/0	2	0	276	4	15	9	35	0
A/1	1	2	7	0	10	15	6	0
A/5	2	2	13	0	14	13	7	0
B/0	341	435	253	165	69	415	57	5
B/1	7	22	68	0	23	74	5	0
B/5	0	6	9	0	7	22	4	0
C/0	6972	3862	277	233	446	1003	68	2
C/1	495	183	156	76	2582	664	82	3
C/5	40	11	61	11	408	254	48	4
D/0	1161	670	259	182	120	966	13	3
D/1	55	34	62	6	258	270	29	1
D/5	4	2	54	2	85	130	27	0

The data in Table 3 show that the degree of infestation in the foodstuff within the different sets of sacks was influenced by the type of sacking and by the quantity of D.D.T. present.

A. *Cotton flour sacks.* The only species of beetle successfully infesting the contents of the untreated sacks was *Ptinus tectus*, and infestation was reduced to a very low level by the impregnation with 1 or 5% of D.D.T.

B. *Jute flour sacks.* All species occurred in relatively small numbers within the untreated sacks, *Tribolium* being the most numerous. Impregnation with 1% of D.D.T. effected a considerable reduction in the numbers of all species and generally increased the proportion of dead beetles. The infestation was reduced to a very low level in those sacks treated with 5% of D.D.T.

C. *Jute bran sacks.* When this type of sacking was untreated, *Calandra* and *Tribolium* were particularly successful in gaining access to the contained foodstuff. Considering the open weave of bran sacks and therefore the short time for which an insect need remain in contact with the D.D.T.-impregnated fibres during passage through the material, the reduction of active infestation brought about by 1 and 5% of D.D.T. is surprisingly large. The high proportion of dead beetles within the sacks is noteworthy; *Oryzaephilus* was the exception in spite of being relatively susceptible to D.D.T. (see Table 4).

D. *Jute grain sacks.* The untreated sacks contained moderate numbers of living beetles of all species, with *Calandra granaria* the most numerous. A reduction to low numbers with a high proportion dead was effected by impregnation with 1% of D.D.T., and a further reduction in infestation resulted from impregnation with 5% of D.D.T.

The degree of infestation in the untreated sacks is clearly related to their closeness of weave. Pl. 3, fig. 1, shows that type A is the most closely woven and type C the

most loosely woven. Types B and D are not easy to classify as the former has more numerous, smaller holes and the latter fewer, often much larger holes. According to Table 3, the materials would be placed in the order A, B, D, C, corresponding to increasing permeability to the insects. The figures for *Ptinus* are exceptional, for this species built up a relatively uniform level of infestation irrespective of the weave.

Infestation has been strikingly reduced by impregnation of all four types of sacking with 1% of D.D.T., and has been still further reduced by impregnation with 5% of D.D.T. Even 5% of D.D.T. on the closely woven cotton flour sacking, however, has not completely protected the contained foodstuff against infestation.

Resistance of beetles to D.D.T. impregnated on sacking

A wide-mouthed glass jar, internally 4·6 cm. in diameter and 6·5 cm. in height, was lined with sacking by fitting a circle 4·6 cm. in diameter on the base and a strip 15·5 cm. in length and 4 cm. in height round the periphery (allowing an overlap of 1 cm.). Twelve jars, fitted with screw tops having metal gauze inserts, were prepared, one for each of the twelve combinations of type of sacking and treatment. Fifty adult *Calandra granaria* were put into each jar and counts of the mortality were made at intervals. When all *Calandra* were dead, they were removed and replaced in turn by *Tribolium*, *Oryzaephilus* and *Ptinus*. A final check was made by retesting *Ptinus* on new sacking to show that the toxicity of the various materials had not altered because of the multiple exposures of insects upon them.

The detailed records showed that the type of sacking had no influence upon the speed of kill. Deaths of insects on untreated sacking did not exceed 6% during the exposure periods required to cause 100% kill on the impregnated sacking, except with *Ptinus*, the exposure of which on impregnated sacking had to be so prolonged that about 50% of the control beetles died from starvation. In the early part of the exposure period, sacking impregnated with 5% of D.D.T. killed beetles more quickly than that treated with 1% of D.D.T., but the times required for 100% kill were not greatly different. The approximate periods of exposure necessary for 100% kill on the 1% D.D.T. sacks only are therefore given in Table 4.

Table 4. *Resistance of beetles kept upon sacking treated with* 1% *of D.D.T.* (25° *C. and* 70% R.H.)

Species	Approx. time for 100% kill (hr.)
C. granaria	150
T. castaneum	140
P. tectus	850
O. surinamensis	100

The results in Table 4 are in agreement with the trend of the data in Table 2, so far as conclusions can be drawn on the relative resistances of the four species. It would appear that the very low percentage kills of *Oryzaephilus surinamensis* recorded in Table 3 were due to an insufficient period of contact with the D.D.T. Experiments are being undertaken to determine the effect on the different species of beetles of various periods of contact with impregnated sacking.

Discussion

When the large-scale experiment was planned, it was anticipated that there would be some points in the results requiring further elucidation, mainly because only one complete examination could be made of each bag of foodstuff. All four species of beetles used in this work can live at least 6 months in the adult stage at 21° C. when allowed to pursue their normal life. In the final examination of the contents of the forty-eight sacks, therefore, beetles which had formed part of the original infestation could not be distinguished from their progeny; nor could beetles which had died naturally be distinguished from those which had died of D.D.T. poisoning. The number of adult insects in the sacks was much lower than had been expected, and it may be that the modes of the emergence curves for the different species had not been reached before sieving was begun. It is difficult to find any other explanation for the small numbers of *Ptinus* and *Oryzaephilus*, especially in the untreated bran sacks, which presumably offered no hindrance to penetration. There is, however, too little detailed information available upon the life histories of the four species of beetles at 21° C. and upon their interaction to attempt an explanation in terms of the capacity for reproduction of the species.

The data obtained for *Ephestia* were disappointingly few. Some small larvae were observed during the intermediate examination, when samples were withdrawn from one-half of the bags, but scarcely any infestation was seen during the final examination. The parent moths were observed to rest on the sacks indiscriminately, but larvae were subsequently noted in the contents of the untreated B, C and D sacks only. The numbers of larvae seen were so small that the results must be regarded as inconclusive, although with a hopeful trend.

The results reported in this paper agree with those of Hayhurst (1945), who worked with miniature sacks impregnated with 0·48–1·63% of D.D.T. by weight. He found that a considerable measure of control was effected by the D.D.T. impregnation, and that *Ptinus tectus* beetles were more resistant than his other two species of test insects, *Calandra oryzae* and *Tribolium confusum*. Good protection of sorghum against insect infestation was also obtained by Nasir (1947) when half-size gunny bags were treated with D.D.T., but the percentage of D.D.T. on the sacks was not stated.

In spite of the lack of a background of detailed information on which some of the experimental data might be explained, a satisfactory answer can undoubtedly be given to the main question, whether impregnation of sacking is of value in preventing insect infestation of bagged goods. Impregnation of sacking with 1% of D.D.T. should markedly reduce the degree of insect infestation of clean bagged goods stored in severely infested stowages. Impregnation with 5% of D.D.T. should restrict infestation to a relatively very low level, and can almost entirely prevent infestation, if the treated material is of sufficiently close weave to afford a considerable mechanical barrier to penetration by insects. It is likely that such penetration as occurred under these conditions in the experiment took place through small faults in the weave or, more especially, through the side seams, which came under strain when the bags were filled.

It must be borne in mind that the risk of infestation to which the experimental

sacks were exposed was extremely severe; under these conditions a 1% impregnation afforded excellent protection, except, perhaps, on the jute bran sacks. The severity of infestation to which bagged goods would be exposed in practice would not usually be so great and a 1% impregnation should prove generally satisfactory. A 5% impregnation should allow a good safety margin to cover loss of D.D.T. by mechanical abrasion during commercial handling, by slow chemical decomposition, and by possible sorption on to the bagged products. The research effort required to prove that an intermediate level of impregnation (below 5% but above 1%) is as efficient in practice as 5% would not be justifiable at present, unless 5% of D.D.T. proves too costly or too difficult to apply, or introduces some toxicological risk. The cost of the treatment will have to be weighed against, on the one hand, the reduction in deterioration of commodities susceptible to insect infestation and the reduction in the numbers of insects carried about in different kinds of transport and, on the other hand, the relatively short life of sacks under certain commercial conditions. Impregnation with D.D.T. from the emulsion led to some shrinkage of the material and the appearance of new sacks was marred. The devising of methods for the satisfactory treatment of different types of sacking with 5% of D.D.T. will no doubt have to be the subject of a special study.

The toxicological risk to man and domestic animals arising from storage and transport of foodstuffs in sacks impregnated with D.D.T. cannot be assessed until data are available upon the quantities of D.D.T. transferred from sacking to different types of foodstuffs. Work is in progress at the Pest Infestation Laboratory upon this latter aspect of the problem.* If the quantities transferred are eventually found to be below the level of any toxicological risk, there now exist sufficient biological data to justify a large-scale trial of the protective value of D.D.T.-impregnated sacking under normal commercial conditions of storage, handling and transport in different parts of the world. If the quantities of D.D.T. transferred are considered to involve risk to the consumer, alternative methods of application might reduce the amounts transferred, or the use of D.D.T.-treated sacks might be restricted to the transport of certain commodities only.

This work forms part of the programme of work of the Pest Infestation Laboratory, and this paper is published by permission of the Department of Scientific and Industrial Research. The original plan of the work was drawn up in consultation with Mr A. F. O'Farrell, then at the Infestation Division, Ministry of Food, and he also arranged for the supply of suitable sacks and foodstuffs.

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* Recent results show that unexpectedly large amounts of D.D.T. can be transferred from impregnated sacking to fatty foodstuffs.

(*Received* 11 *October* 1947)

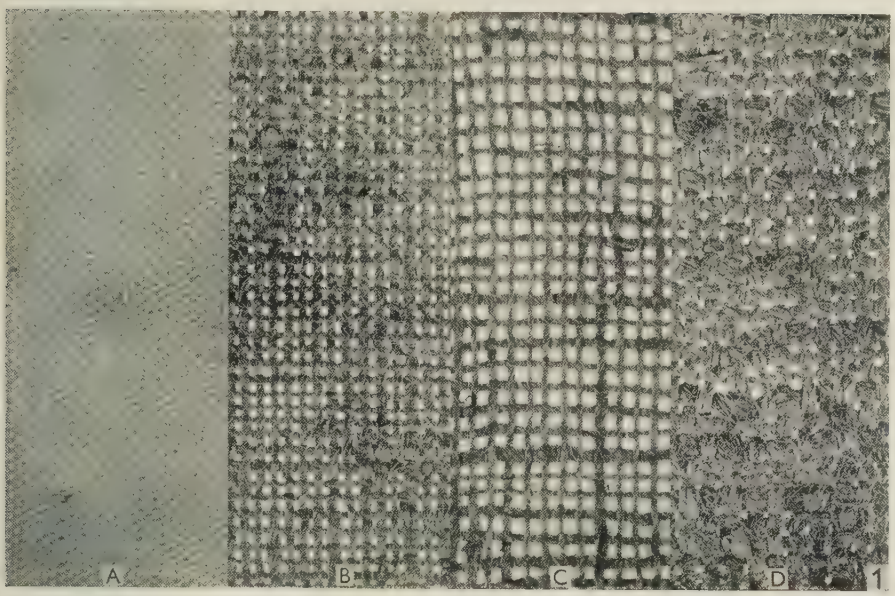

Fig. 1. The four types of sacking. (Natural size.)

Fig. 2. The four types of completed sack.

VERTICILLIUM WILT OF THE HOP (*HUMULUS LUPULUS*)

III. A STUDY OF THE PATHOGENICITY OF ISOLATES FROM FLUCTUATING AND FROM PROGRESSIVE OUTBREAKS

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(With Plate 4)

An account is given of the methods used to determine the relative pathogenicity of isolates of *Verticillium albo-atrum* from fluctuating and from progressive outbreaks of the wilt disease of hops. Isolates from fluctuating outbreaks were less pathogenic to young hop plants than those from progressive outbreaks, and it is suggested that this is the main cause of the differences in symptom incidence and intensity between the two types of outbreak in the field.

Earlier work by one of the writers (Keyworth, 1942) showed that outbreaks of *Verticillium* wilt of the hop could be grouped on field symptoms into two types, called *fluctuating* and *progressive*. As their names indicate, the main distinction between these two types was that one fluctuated in intensity from year to year, whereas the other became progressively worse. Harris (1936) reported that in the (fluctuating) outbreaks that he studied, the annual variation in the number of plants displaying symptoms and the severity of these symptoms was related to the moisture content of the soil in the summer. In such outbreaks, plants showing symptoms in one season often appeared healthy next year and vice versa. No such correlation between soil moisture and disease incidence or severity was found by Keyworth (1942) in his study of progressive outbreaks, and affected plants almost invariably died within 1 or 2 years.

Besides these basic differences in annual disease severity, other differences between the two types of outbreak were noted, principally in the type of symptoms displayed and the distribution of the visibly affected plants. In fluctuating outbreaks, usually single stems on widely scattered plants showed a somewhat diffuse leaf yellowing late in the season, sometimes followed by wilting. The inner wood in such stems was brown, but new wood often developed causing a characteristic swelling of the stem with a corky appearance of the bark. In progressive outbreaks, on the other hand, often all the stems on affected plants showed severe symptoms early in the season, culminating in the death of the bines without prior thickening, and with browning of the whole wood cylinder. The disease spread from scattered foci and thus infected plants tended to occur in well-defined groups.

The individual plant symptoms suggested that, in fluctuating outbreaks, the

* Resigned.

host/parasite relationship was such that the plant was able to withstand the effects of the fungus for a long period—possibly as a result of the production of fresh vascular tissue already described—whereas in progressive outbreaks the fungus rapidly killed the bines.

Within each of the two main types of outbreak already described, a further graded classification was possible. Thus in some fluctuating outbreaks the only symptom was the thickening of the bines on some of the plants, whereas in others some wilting was invariably seen. Progressive outbreaks also showed differences from farm to farm in the rate of spread of the disease. The variations within each type, however, were never so clear or readily classifiable as those between the two types, which all available field evidence suggested were fundamentally distinct.

A study of cultures of *Verticillium albo-atrum* from a number of outbreaks of each type failed to reveal any consistent morphological differences between them, and, although it was then suggested that the severity of the disease might be correlated with soil conditions, the causes of the differences between the two types of outbreak remained obscure.

The Ministry of Agriculture scheme for the certification of wilt-free hop gardens which was started in 1943 revealed a large number of fluctuating outbreaks which were so mild that they had not been noticed by the growers. With this information, and added notifications of new progressive outbreaks, it was possible to map the distribution of some 130 outbreaks in south-east England. This demonstrated that whereas the fluctuating outbreaks were fairly widely distributed, the progressive outbreaks were, with a few exceptions, confined to an area around Paddock Wood in Kent (Keyworth, 1945). As the number of progressive outbreaks had increased year by year in this area, it seemed possible that this form of the disease was spreading independently of the fluctuating type. Thus although the earlier work had not shown any consistent morphological differences between isolates, this later information suggested that the two types of disease were in fact caused by different strains of *V. albo-atrum*.

The present investigation was therefore started in 1944 to study the pathogenicity of isolates from a number of outbreaks of both types. As previous work (Keyworth, 1942) had shown that hops could be successfully inoculated with progressive wilt by planting them in soil to which naturally infected hop stems had been added, the method of soil inoculation was chosen for the main experiments, although direct plant inoculations were also made.

Soil inoculation experiments

1944. As insufficient pure culture inoculum was available in 1944, two preliminary experiments were conducted using naturally infected hop stems collected in the previous autumn from three gardens with progressive, and three with fluctuating wilt which were selected as representative. The stems were chopped into 1 in. lengths and stored dry over the winter.

The two experiments were identical in lay-out, but one was conducted on the sandy-loam soil of the East Malling Research Station, and the other on the heavy silt loam at Beltring near Paddock Wood. The methods used in each experiment were as follows:

Twenty plants of the highly susceptible Fuggle variety were grown in soil inoculated with each of the six types of stem and forty similar control plants were grown in uninoculated soil. The soil was inoculated by placing about 10 g. of stem in each planting hole ($8 \times 8 \times 8$ in.) and covering it with about 1 in. of soil before inserting the plant. The treatments were randomized in five-plant plots to eliminate chance soil effects. The plots were 1 yd. apart in rows 4 ft. apart, the plants being spaced 18 in. apart in the plots.

Wilt symptoms were noted on some of the plants in June and records were taken at approximately 3-week intervals thereafter. For the final symptom records the plants were grouped into four categories: (*a*) *Severe wilt*—plants dead or nearly so, sometimes with one or more small normal stems growing late in the season. (*b*) *Wilt*—most of the stems showing severe symptoms but few of the stems dead. (*c*) *Mild wilt*—a few leaves on some of the stems showing yellowing and partial desiccation. Such leaves were usually near the bases of the stems but occasionally most of the leaves on a stem were affected. (*d*) *Apparently healthy*—no leaves showing obvious yellowing. The final records for both experiments are given in Table 1.

TABLE 1. 1944 *soil inoculation experiment*

		East Malling				Beltring			
Source of inoculum	No.	Severe wilt	Wilt	Mild wilt	Apparently healthy	Severe wilt	Wilt	Mild wilt	Apparently healthy
Progressive outbreak	1	9	9	2	0	20	0	0	0
	2	11	7	2	0	18	2	0	0
	3	15	5	0	0	16	4	0	0
Fluctuating outbreak	1	0	1	13	6	0	5	13	2
	2	0	0	13	7	0	4	15	1
	3	0	0	15	5	0	9	9	2
Control		0	0	0	40	0	0	0	40

These results indicate that the addition to the soil of stems from progressive outbreaks caused severe wilt in a large number of indicator plants, at both sites, whereas stems from fluctuating outbreaks caused mainly mild symptoms. This suggested that the strains of *V. ablo-atrum* in the two types of stems were of differing virulence. Photographs of typical progressive, fluctuating and control plots are shown in Pl. 4. It will further be noted that there was a higher proportion of severe wilt and wilt at Beltring than at East Malling, but that the same differences between the treatments were apparent at both sites.

1945. As the 1944 experiments had used naturally infected stems as inoculum some doubt still remained that the effects observed were due solely to the strains

of *Verticillium* presumed to have been present in the stems. In the 1945 experiments therefore, three types of soil inoculum were used, as follows:

(*a*) Naturally infected stems.

(*b*) Cultures of *V. albo-atrum* grown on sterilized hop stems from portions of naturally infected stems.

(*c*) Similar cultures grown from previously isolated and identified agar cultures.

Soil inoculum type (*b*) was included in order to guard against any change in virulence which might have occurred when the fungi were grown in agar culture.

The soil inocula were prepared from two progressive and two fluctuating outbreaks (sources 1 and 2 of each type used for the 1944 experiments), and the experimental lay-out and methods of soil inoculation were similar to those used in 1944, fresh sites being chosen in the same fields at the two localities.

Symptoms were again first noted some 8 weeks after the start of the experiments. In this season, however, many of the plants, including the controls, showed leaf scorch, and it was thus impossible to diagnose mild wilt accurately. For this reason, only the three symptom categories *severe wilt*, *wilt* and *apparently healthy* were used. After the final symptom record, the stems of all the plants in the experiment at East Malling were cut 2 in. above ground-level in order to determine whether they contained brown wood and could thus be regarded as infected. (Isolations from a representative number of such bines confirmed that this was the case.) The records taken at East Malling are given in Table 2.

TABLE 2. 1945 *soil inoculation experiment at East Malling*

Source of inoculum	Type of inoculum	No. of plants inoculated	No. containing brown wood	No. with severe wilt	No. with wilt	No. apparently healthy
Progressive 1	*a*	20	20	20	0	0
	b	19	19	18	0	1
	c	20	19	17	2	1
Progressive 2	*a*	19	19	19	0	0
	b	19	18	16	1	2
	c	20	18	17	1	2
Fluctuating 1	*a*	20	18	0	0	20
	b	20	20	0	2	18
	c	20	18	0	1	19
Fluctuating 2	*a*	20	20	0	0	20
	b	20	20	0	0	20
	c	20	20	0	1	19
Control		120	5*	1	0	119

* All the infected controls were adjacent to plots infected with 'progressive' inoculum.

These results indicate that:

(*a*) Almost all the inoculated plants became infected.

(*b*) Almost all the plants inoculated with strains of *V. albo-atrum* from progressive outbreaks showed severe wilt, and most of those inoculated with strains from fluctuating outbreaks showed little or no wilt.

(*c*) The type of inoculum used did not affect the intensity of the symptoms produced by any strain.

(*a*) Plot inoculated with stems from progressive outbreak.

(*b*) Plot inoculated with stems from fluctuating outbreak.

(*c*) Control plot.

Similar records were taken on the plants grown at Beltring, but as brown wood records are not available the number of infected plants is unknown. Symptom records are shown in Table 3.

TABLE 3. 1945 *soil inoculation experiment at Beltring*

Source of inoculum	Type of inoculum	No. of plants inoculated	No. with severe wilt	No. with wilt	No. apparently healthy
Progressive 1	*a*	20	20	0	0
	b	20	20	0	0
	c	20	17	0	3
Progressive 2	*a*	20	20	0	0
	b	20	20	0	0
	c	20	19	0	1
Fluctuating 1	*a*	19	0	1	18
	b	20	0	0	20
	c	20	0	0	20
Fluctuating 2	*a*	20	1	0	19
	b	20	0	1	19
	c	20	0	0	20
Control		118	1	0	117

The wilt records at Beltring were thus similar to those at East Malling. Both experiments show the considerable differences in wilt incidence caused by the inocula from fluctuating and from progressive outbreaks, and show that this effect was the same on both types of soil.

DIRECT PLANT INOCULATIONS

In June 1944 a number of Fuggle hop plants, some at East Malling and others at Beltring, which had been planted in the previous March, were inoculated with isolates of *V. albo-atrum* from fluctuating and progressive outbreaks in two ways: (*a*) by inserting a portion of agar culture into a scalpel wound, made in the stem at ground-level, pressing back the flap of tissue and covering the base of the plant with soil; (*b*) by injecting about $\frac{1}{2}$ c.c. of a concentrated spore suspension into a stem 3 ft. from the ground. Control plants were wounded, but not inoculated or were injected with water only.

Although many of the inoculated plants became infected and developed brown wood, no severe wilt symptoms were seen on any plant, and there was thus no differentiation between the fluctuating and progressive strains used.

In order to test whether this effect was due to a loss of pathogenicity of the progressive strains in culture an experiment was carried out in 1945 on the same lines but using portions of naturally infected stem and spores from such a stem as inoculum as well as agar cultures and spores from such cultures. The inoculations were again made in June on plants established in the previous March and were carried out in the same fashion as those in 1944.

Probably because of the dry weather in June, a large number of the wound inoculations failed to infect the stems, but the hypodermic injections produced a high proportion of infections. The results of the inoculations at both sites are shown in Tables 4A and 4B.

TABLE 4A. *Wound inoculations*, 1945

Fungal strain	Type of inoculum	No. of successful inoculations	No. showing obvious wilt
Progressive	Culture	11	4
	Stem	10	5
Fluctuating	Culture	4	1
	Stem	9	2

TABLE 4B. *Spore injections*, 1945

Fungal strain	Source of spores	No. of successful inoculations	No. showing obvious wilt
Progressive	Culture	36	4
	Stem	39	8
Fluctuating	Culture	38	4
	Stem	35	3

These results show that although there was slightly more wilt in the plants inoculated with the progressive strain than in those inoculated with the fluctuating strain the differences were not comparable with those shown by the method of soil inoculation. It is also apparent that the type of inoculum did not affect this result.

A further small-scale trial was therefore conducted in 1946 to determine whether this anomalous result was due to the later time of inoculation or was connected with the fact that the fungus was inserted into the stems and not into the roots. In this experiment, groups of four plants were inoculated with two progressive and two fluctuating strains of *V. albo-atrum* by introducing portions of (pure) fungal culture on hop bine, or spores from such cultures, into the roots and stem bases of Fuggle hop sets before these were planted in March. Wounded but uninoculated controls were also grown. The experiment was conducted at East Malling and observations made throughout the season. All the treatments gave a high proportion of infected plants (as indicated by the brown-wood test), but no striking differences in wilt incidence were noted between the root and stem, or wound and injection inoculations. Of the total of thirty-two plants inoculated with progressive strains (twenty-nine of which became infected), seventeen were severely wilted, two showed wilt and seven were mildly wilted. Of the same number of plants inoculated with fluctuating strains, twenty-nine became infected, none was severely wilted, one showed wilt and nineteen were mildly wilted.

These results are thus in agreement with those obtained in 1945 by soil inoculation and provide further confirmation of the differences in wilt incidence caused by the fluctuating and progressive strains used.

Conclusions

The soil inoculation experiments and the final direct inoculation experiment demonstrate that the fluctuating strains used were less pathogenic to young hop plants than the progressive strains. As already described, the outbreaks from which these strains were isolated were chosen as being representative of many others of each type, and it is thus a valid assumption that the main distinction between fluctuating and progressive outbreaks in established hop gardens lies in the strain of *V. albo-atrum* involved. It has already been noted, however, that progressive and fluctuating outbreaks of varying degrees of severity can be found. The differences in the severity of symptoms shown in the experiments at Beltring and East Malling suggest that soil factors may be partly responsible for this effect but it is of course possible that further strains of *V. albo-atrum* exist.

Grateful thanks are due to Dr R. V. Harris, who has not only contributed much to the work by his helpful suggestions, but has also freely placed his considerable experience and records of fluctuating outbreaks at the writers' disposal. The writers are also indebted to Mr S. C. Pearce for his advice on the lay-out of the experiments and to Miss M. Bennett and Miss M. M. Hitchcock who undertook much of the recording.

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(*Received* 11 *August* 1947)

STUDIES ON THE IMPORTANCE AND CONTROL OF POTATO VIRUS *X*

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(With Plate 5)

The success of seed certification schemes in controlling the aphis-transmitted viruses that cause leaf roll and rugose mosaic has greatly increased the relative importance of potato virus *X*, in spite of the value of such schemes in reducing the prevalence of the most virulent strains. Virus *X* occurs throughout commercial stocks of most varieties and is responsible for many of the uncertainties and difficulties encountered in field inspections, for the production of leaf symptoms by many strains depends on the weather. Also, the predominating virus strain in a stock may change from season to season.

Methods of testing for the presence of avirulent strains in potatoes are described. These include transmission to suitable indicator plants, plant-protection tests, and serological methods. The virus content of different parts of the plant varies widely, but is everywhere high enough for transmission to indicator plants at all times of the year. The virus content of immature tubers is too low to give a precipitin reaction, but extracts from developing sprouts contain nearly as much virus as those from foliage, and can be used reliably.

Infection of virus-free stocks of the varieties Majestic and Arran Banner with four different strains of virus *X* reduced their yields by amounts varying from 5 to 24%. Scotch Stock Seed Majestic, however, cropped better than the virus-free seed, and the need for considering additional factors to freedom from viruses when selecting clonal lines for propagation is stressed.

Virus *X* was not transmitted by cutting healthy tubers with a knife previously used to cut infected ones, and cut tubers whose storage parenchyma was rubbed with infective sap gave rise to virus-free plants. Infection occurred when sprouts were rubbed with infective sap, and the virus also spread from infected to healthy sprouted tubers stored in the same sack.

Spread of the virus under natural conditions is slow, and it is concluded that the maintenance of virus-free stocks under commercial conditions is feasible, provided that precautions are taken to prevent contact with infected plants. The relative importance of volunteer plants and other sources of infection is discussed, and methods of testing the virus-free stocks are suggested.

During recent years the relative importance of different potato viruses in Britain has changed strikingly. From being of little importance compared with either leaf-roll virus or virus *Y*, virus *X* now probably causes more loss of crop than the other two combined. The change reflects the large measure of success achieved by potato seed certification schemes in controlling the two aphis-transmitted viruses without similarly reducing the prevalence of virus *X*, and was probably inevitable from the way in which such schemes originated and have been operated. The control of virus *X* necessitates measures additional to field inspections of crops in districts where conditions are unfavourable for the activities of potato aphides. It is the purpose of this paper to describe observations and experiments dealing with some

of the problems raised by the almost universal occurrence of virus *X* in commercial potato stocks and by the adoption of measures needed for its elimination.

Seed certification and potato virus *X*

The economic importance of a disease depends not only on its severity but also on its prevalence. Severe diseases are obvious and their importance can be easily overestimated, whereas less obtrusive but more prevalent diseases may cause more loss and pass unnoticed. This is now largely true of potato-virus diseases in Britain where emphasis is mainly laid on leaf roll and rugose (severe) mosaic, caused by virus *Y*. Both give rise to pronounced symptoms in the haulms and reduce the yield of individual plants by 50% or more. Between them they are responsible for the drastic decline in the cropping power of potatoes when grown for a few years in districts where potato aphides are active. By comparison, plants with mild mosaic, the disease most commonly caused by virus *X* in the field, suffer little, and while leaf-roll virus and virus *Y* were prevalent, virus *X* was unimportant. Now, however, as a result of improved standards of health demanded by seed certification schemes and because ware growers have generally adopted the practice of obtaining new stocks of certified seed at frequent intervals, leaf roll and rugose mosaic have become much less prevalent. It is rare to see crops containing more than 10% of the plants with these two diseases, and their total effect on yield is now slight. Their cost is to be measured mainly as the cost of their control, i.e. the difference in price to the ware grower between new seed and the seed-sized tubers from his own crop, rather than by any great loss of crop.

The success of the seed certification schemes in controlling leaf roll and rugose mosaic depends on two facts: (1) thorough roguing is possible because infected plants are readily recognized by field inspection, and (2) the diseases are spread by aphides and their rate of spread can be greatly reduced by growing seed stocks in districts unfavourable for the activities of potato aphides. Neither of these facts holds for potato virus *X*, and the seed schemes have therefore had no similar success in reducing its prevalence. The virus is as common, and probably spreads equally freely, in seed-growing districts as elsewhere, but spread is of little importance because commercial stocks of most varieties are already heavily infected, many to the extent of every plant. The only exceptions are a few varieties, such as King Edward, which are hypersensitive to most strains of the virus. In Britain alone at present rates of production there are annually about 700,000 acres of potatoes infected with virus *X*, which at a conservative estimate must be reducing yield by more than 500,000 tons. In other countries the position is even worse, for all the widely grown varieties are 100% infected.

Although seed certification schemes based on field inspections have not reduced the prevalence of virus *X*, they have undoubtedly had an important effect in preventing the losses from being even greater than they are. Virus *X* exists in many strains that differ from one another in the type and severity of symptoms they cause.

Strains that cause obvious symptoms are easily observed and infected plants can be removed, but those that cause slight symptoms are undetected and remain in the stocks. Hence the inspection schemes have exerted a strong selective action on the different strains of virus *X*, operating against virulent and favouring avirulent ones. As a result, the commonest strains in a variety are those that cause only slight symptoms. This selection has in itself been a useful control measure, but the process has now probably achieved as much as it can in reducing losses.

The presence of virus *X* in seed stocks is the main cause of uncertainties and difficulties that arise in field inspections, for mild mosaic is included in the certification schemes but its identification is unreliable and erratic. Infected plants may show definite mottling early in their growth, but be symptomless later, and mosaic symptoms are obscured and infection is masked by a period of high temperature. Also, a mottle that is detectable on a dull day may be invisible in bright sunlight. Thus one season a stock may be placed in one seed category and the next in another, depending on the stage of growth of the plants or the weather prevailing before or at the time of inspection. Symptoms of mild mosaic are also affected by manuring, being masked by heavy dressings of nitrogenous fertilizers. Infection is particularly difficult to detect when all the plants in a stock are uniformly infected with the same strain of virus, for plants that would be noticeably pale or mottled when compared with virus-free plants appear normal when all are similar. Unless there are quite characteristic lesions, the standard for comparison in field inspections tends to be the healthiest-looking plants in the crop being examined. Hence a stock which has most of its plants infected with a mild strain but has a few infected with more virulent ones is more easily identified as infected, and may easily be placed in a lower category than a stock uniformly infected with the more virulent strain. It is also common for stocks certified as free from mild mosaic in one year to contain appreciable numbers of plants showing mild mosaic, or even severe mosaic and necrosis, in the next year. This is usually attributed to current season infection, but is more often the result of the segregation of a more virulent strain from the mild one present in most of the stock.

To prevent the losses caused by the almost universal occurrence of virus *X* and to increase the reliability of field inspections, the elimination of the virus from seed stocks is essential. There are two ways in which this might be done. One is by breeding new potato varieties that are either immune from or hypersensitive to the virus. Immunity is preferable to hypersensitivity and seems a practical aim since the production of a seedling (U.S.D.A. 41956) that has proved to be immune from all strains of virus *X* yet studied. The value of hypersensitivity is already well established by the behaviour of such varieties as King Edward, for stocks of these are rarely found infected with virus *X*; field inspection is simpler and more reliable, and the highest seed categories are attained with greater ease than with varieties such as Majestic. However, before this method of control can become general, new varieties will need to be produced at least equal in cultural and culinary qualities to

those now favoured by growers. The general introduction of such varieties would be a great advance, but too much reliance should not be placed on it alone as a permanent solution to the problem. Virus *X* is a labile entity, and strains already occur to which King Edward is not hypersensitive (Bawden & Sheffield, 1944), and the possibility of other strains arising in the future to which such varieties are susceptible and tolerant must be entertained. The second method of eliminating virus *X* is already being attempted with existing varieties; it entails, first, the discovery of virus-free plants and, secondly, the multiplication of these in their virus-free state to quantities sufficient to replace existing commercial stocks.

Testing for virus *X*

The symptoms caused by virus *X* on potatoes range from a lethal top-necrosis, through severe crinkles with necrotic spotting and interveinal mottling, to mild mosaic and no perceptible leaf symptoms. Most of these types of symptom have been described in detail by Salaman (1938) and need no further consideration here, except to stress the variability of symptoms that may be caused by the same strain in different varieties or by different strains in the same variety. The term virulence can only be used accurately in conjunction with specified virus-host combinations, for any one strain may be virulent towards one potato variety and avirulent towards another. The range of symptoms produced by strains of virus *X* in different potato varieties is so great that, from appearance alone, it is often impossible to determine whether a diseased potato is infected with a strain of virus *X* or with some unrelated virus. Some strains we have obtained from naturally infected potatoes cause mosaic and necrotic spotting in Majestic leading to a leaf fall that resembles leaf-drop streak caused by some strains of potato virus *Y* (Bawden & Kassanis, 1946) (Pl. 5, fig. 1). These necrotic plants, however, do not show the veinal streaks characteristic of virus *Y* infections, and the severe leaf drop usually occurs only early in the year, the symptoms later consisting largely of necrotic spotting and mosaic. The certain diagnosis of infection with virus *X* can be made only as a result of immunological tests. Two methods are available: (1) to determine whether the virus reacts specifically with an antiserum made against a type culture of virus *X*, and (2) to determine whether the unknown virus and a type culture of virus *X* mutually protect plants against each other (Pl. 5, fig. 2). Strains of virus *X* that produce no necrotic lesions in a potato variety sometimes fail to protect completely against strains that do, especially if the latter are introduced by grafting. Hence the protection test is more reliable if made with tobacco or *Datura stramonium*, and if infections are made by inoculation of sap rather than by grafting.

For the practical purpose of establishing and maintaining virus-free potato stocks, tests are unnecessary for virus strains that cause definite leaf symptoms. Plants infected with such strains can be identified from field inspections and their control presents no problems. Only the strains that cause slight or no leaf symptoms in potatoes call for special control measures, but these are now the most prevalent.

Fortunately, there are simple and reliable tests for their presence. Many such strains produce clear leaf symptoms in tobacco and *D. stramonium* and can be detected by inoculating young seedlings of these plants with sap from potatoes. In our experience, *D. stramonium* is preferable to tobacco, for it is more susceptible to infection and reacts clearly with some strains of virus *X* that produce indefinite symptoms in tobacco. Tobacco seedlings react better in winter than in summer, and in the summer their sensitivity is increased if they are raised under shade. Virus *X* is readily transmitted merely by lightly rubbing the leaves of test plants with sap from macerated infected potatoes, but transmission is more certain if the leaves to be rubbed are first dusted with kieselguhr or 400-mesh carborundum. The virus content of different parts of potato plants varies, but is everywhere sufficiently high for successful transmission, especially if an abrasive is used. When tests are made from dormant tubers, however, it is advisable to take inoculum from cortical tissues containing 'eyes' rather than from storage parenchyma only. The symptoms produced by different strains of virus *X* in tobacco and *D. stramonium* range from severe necrosis and ringspot, through mosaics of differing intensities, to vague and barely perceptible mottlings. A positive reaction in the inoculated seedlings condemns the tested potato as infected, but a seemingly negative result cannot be taken as certainly implying that the potato was virus-free. To confirm that the inoculated seedlings are free from virus *X* and not infected with strains that they can carry without showing symptoms, a further test is needed. This is conveniently done by testing the reactions of the seedlings to a strain that produces severe symptoms. Three weeks or so after the first inoculation, all the healthy-looking seedlings, together with some previously uninoculated controls of the same age, should be inoculated with the virulent strain. If they are still susceptible and react in the same way as the healthy control plants, then it can be safely assumed they are uninfected, whereas if they are protected against the effects of the second inoculation then they were infected with an avirulent strain from the potato.

As an alternative or additional test to inoculation to tobacco and *D. stramonium*, serological methods can be employed. These give results much more quickly than inoculation tests, are much cheaper, and detect the virus in symptomless carriers as easily and positively as in those showing symptoms. Of the methods that can be employed one or other variant of the precipitin test is most suitable for use on any large scale. Virus *X* is a good antigen and antisera with high precipitin titres are produced when rabbits, guinea-pigs or other suitable animals are injected with it. By comparison with the virus, normal constituents of solanaceous plants seem to be weak antigens and antisera prepared by the injection of clarified sap from infected plants contain predominantly antibodies to the virus. In our experience, antisera prepared in this way when used at a dilution greater than 1 in 20 have never precipitated specifically with sap from healthy plants. However, to avoid complications from the possible antigenicity of normal plant constituents, it is preferable to prepare antisera by injecting purified preparations of virus *X*. If infective sap must be used,

then the antisera should be fully absorbed with sap from healthy plants before it is used for testing for the virus. Severe reactions in the injected animals are sometimes caused by intravenous injections of sap, and if sap is used it should be injected into the peritoneal cavity rather than intravenously. Six injections of 5 ml. of sap at 5-day intervals usually produce an antiserum with a precipitin titre greater than 1 in 1000. Purified virus preparations can be injected safely into the veins, and four injections each of 2 mg. of virus usually suffice to produce a good precipitating antiserum. An antiserum prepared against one strain of virus *X* precipitates with all other strains of virus *X*, but with no other virus.

In testing for the presence of virus *X* with antiserum, crude sap expressed from macerated tissues can be used, but control tests with normal serum and saline must also be made because crude sap may precipitate spontaneously. However, the specific reaction is usually rapid, positive results being obtained within a few minutes of mixing antiserum with sap from infected leaves. The reaction with crude sap is also easy to see, for a bulky precipitate is obtained containing the plastids and other suspended green material. Tests can be made either by mixing 0·5 ml. or more of sap with an equal volume of suitably diluted antiserum in Wassermann tubes, as described by Chester (1937), or by mixing drops of sap and antiserum on glass slides, as in the agglutination tests described by van Slogteren (1945). With a suitable tool for crushing and extracting small volumes of sap, the agglutination method is very convenient and large numbers of plants can be examined in a short time. There is an optimal ratio of virus to antibody that gives the most rapid precipitation, and precipitation may be wholly inhibited if virus is present in great excess. In working with these methods, therefore, which involve only one dilution of sap and antiserum, it is advisable to use fairly high concentrations of antiserum. The optimal concentration of serum for use will vary with different sera, depending on their antibody content, and is a matter to be decided by experiment.

Variations in virus content

The results of such tests with crude sap are reliable enough for the immediate detection of virus in most infected plants. The uncertainties caused by spontaneous precipitations, or by possible failures of precipitation caused by an excess of virus, can be eliminated by more refined methods. The simplest is to clarify the sap before testing by heating to 58° C. and centrifuging. This treatment gives a stable, clear fluid, which with antiserum gives a characteristic fluffy type of precipitate. If such fluids are tested over a range of dilutions, highly diluted antisera can safely be used and the method can also be employed to assess the quantity of virus present. This is the method we have used most, 1 ml. of antiserum at a constant dilution (usually 1 in 200) being added to each of a series of tubes containing 1 ml. of clarified sap at different dilutions. Immediately after adding the antiserum, the tubes are placed in a water-bath at 50° C. Precipitation occurs within a few minutes in tubes in which the ratio of antibody to virus is near to the optimum, and more slowly in others. The

highest dilution of sap at which a visible precipitate can be obtained (the precipitin titre) can be used as a convenient measure of the virus content.

Precipitin reactions can be obtained with sap expressed from all parts of the potato plant except from the storage parenchyma of tubers, but there are considerable variations in the virus content, and so in the strength of the reaction, of sap from different tissues. The virus content also varies with different strains of the virus. We have determined the annual fluctuations of virus content in Stock Seed Majestic infected with a strain of virus *X* that causes a barely perceptible mottle. Sap taken from tubers soon after harvest contained too little virus for the precipitin test to be used reliably; some tubers failed to react at all, whereas sap from the peel of others reacted when undiluted. Three months after harvest, before sprouting was significant, the virus content of extracts from the peel had increased so that precipitin titres of 1 in 4 or 1 in 8 were usual, with titres twice as great if only 'eyes' and the immediately adjacent tissue were used; extracts from the tuber parenchyma failed to react, though they contained some virus, for they were infective. With the start of sprouting, the virus content rose rapidly in the sprouts, and sap from sprouts 1 cm. or more long gave precipitin titres of between 1 in 80 and 1 in 320, although extracts from the storage parenchyma still failed to give a reaction. The virus content of sap from sprouts at this time is nearly as high as that of sap from leaves, for the precipitin titre of the latter remained fairly constant throughout the growing season and never exceeded 1 in 640. These results show that in testing potatoes for the presence of virus *X* there is no need to wait until plants have foliage. Sprouts supply a reliable source of material for both serological and infectivity tests, which can equally well be made during the winter from sprouted tubers as from growing plants.

The virus content attained by different strains of virus *X* in the foliage of potato plants varies significantly. In one comparison in which Majestic plants infected with four different strains were grown under glass, precipitin titres were 1/1280 with a strain producing a bright yellow mottle, 1/640 with potato virus *B*, which produces slight or no symptoms, 1/320 for a strain derived from Stock Seed Majestic and producing no leaf symptoms, and 1/160 for the fourth, which produced a definite but mild mosaic. Differences of the same order have also been found between the precipitin titres of sap from plants infected with different strains when growing in the open. No general statement can be made about correlation between symptoms and virus content, for the virus content of plants infected with different strains that cause slight or no leaf symptoms varies considerably, but the highest titres we have obtained from potatoes have been from plants showing pronounced leaf symptoms, either a bright interveinal mottle or mottle with necrosis and deformity. Similar differences are also found when strains are propagated in different hosts. In tobacco the virus content achieved is only slightly higher than in Majestic potatoes, and again severity of symptoms is no sure guide of virus content. Of two strains producing almost identical ringspot symptoms, one

(Salaman's X^S) gave a precipitin titre of 1/320 and the other a titre of 1/1280, whereas of two strains producing mosaic symptoms only, one a bright yellow mottle and the other a diffuse, faint mottle, both gave a titre of 1/640. In tomato all the strains reach higher concentrations, clarified sap from plants with these four strains giving titres of between 1/1000 and 1/5000. With some strains the virus content of tomato sap is such that clarified sap shows the phenomenon of anisotropy of flow strongly when it is shaken in narrow tubes between crossed polaroid plates.

The results of our quantitative tests for virus *X* in different parts of potato plants suggest an explanation for the need of annual roguing with commercial stocks of such varieties as Majestic, in which plants with definite leaf symptoms occur each spring though none was present in the previous autumn. Often such diseased plants are not uniformly affected, and single shoots may show pronounced symptoms, either mosaic or necrosis, while most of the plant appears normal or shows only a faint mottle. Tests made from such plants have demonstrated that all the shoots are infected with virus *X*, but that those with different symptoms contain strains of different virulence. The production of severe symptoms is not produced by current season infection of single shoots with virulent strains from outside sources, because we have occasionally noted the same effect under glass (Pl. 5, fig. 3). Similarly, in experiments under glass in which we have cut tubers into pieces bearing single 'eyes', plants showing widely different symptoms have occasionally developed from different pieces of one tuber. Most plants produced in this way look normal or show only a faint mosaic, but occasionally there is one with a bright mosaic or a severe necrotic spotting. Transmissions from the progeny of such tubers to tobacco or *D. stramonium* have shown that all are infected with virus *X*, but those with pronounced symptoms contained strains that cause ringspot or bright mosaic symptoms in the test plants, whereas the others contained strains that cause only a mild mosaic. When transmissions were made to healthy Majestic, inoculum taken from plants with different symptoms reproduced the characteristic differences, and, provided bulk transfers are made, these reactions remain fairly constant. Nevertheless, such plants usually contain a mixture of virus strains, a fact immediately demonstrable by applying some suitable selective treatment, such as taking inoculum from single local lesions or from minute portions of leaf tissue as described by Salaman (1938).

The production of symptoms in some plants, or in individual shoots of some plants, of stocks of potatoes that were previously symptomless, is a result of natural segregation of virulent strains from mixtures in which avirulent strains predominate. Opportunities for the production and selection of such strains are afforded by the large annual fluctuations in the quantity of virus *X*. During the growing season, to account for the high virus concentration reached throughout the haulm, there must be a vast multiplication of virus particles, and this supplies chances for numerous mutations. The existence of most mutations will remain undetected, because they will form only a small fraction of the total virus population, and any specific effects

they might have will be overshadowed by those of the numerically predominating parent strain. Our tests on immature tubers show that there is no great accumulation of virus in them as a result of mass movement from the haulms to the tubers. Instead, it seems that there is a drastic fall in virus population at the end of the growing season when the tops die, and infection of the tubers occurs by relatively little virus, which is mainly established in or near the 'eyes'. This supplies a mechanism capable of selecting mutants in a relatively pure state free from the predominating strains, and of altering the relative proportion of virus strains present in a potato stock from year to year. There seems to be little multiplication or free movement of virus through the body of the tuber, for increase in virus content occurs only when the 'eyes' begin to sprout and then only in and around the sprouts. Thus the virus strains that first get established in the developing 'eyes' will be able to multiply free from competition from other strains and will be those that will predominate in the shoots that grow from those 'eyes'. Strains that multiply most rapidly and move most freely will have obvious advantages over others, but even with no differences in such characters of survival value, there will still be opportunity for segregation. The infection of 'eyes' by particles at random will exert a selective action similar to that operating when single local lesions are used as sources of inoculum, and the numbers of 'eyes' becoming infected with different strains will depend on the proportions in which the strains occurred mixed in the parent plant. Most 'eyes' will, of course, contain virus resembling the predominant strain of the previous year, but if a virulent one was present to the extent of 1% (a proportion that would produce no obvious results, as we have shown by inoculating healthy plants with mixtures of sap containing different strains), then it could be expected to occur and produce its characteristic effects in about 1% of the new shoots. An acre of potatoes may contain over 100,000 separate shoots, and Stock Seed certificates allow a maximum of four plants showing severe symptoms, whatever virus they may be caused by. Our results show that strains of virus *X* causing severe symptoms usually multiply more freely than those that are carried, and a very high mutation rate is not needed to explain the difficulties of Stock Seed growers in reaching the necessary standard when working with *X*-infected stocks.

The effect of virus *X* on yield of potatoes

It is impossible to give any precise estimate of the losses caused by virus *X*, for there is little information on the relative prevalence of strains of different virulence. There is no doubt that virulent strains have a serious effect on yield, for it is obvious at sight, but there is a dearth of quantitative data. Scott (1941) in Scotland compared the yields of symptomless Majestic plants with those of plants showing 'negligible mottle', 'mild mosaic', 'border-line severe mosaic', and 'severe mosaic', and found a close correlation between the appearance and yield. The identity of the viruses concerned is uncertain, but it is probable that the first three symptoms were

caused by infection with increasingly virulent strains of virus *X*, and plants showing these yielded respectively 13, 34 and 43% less than the symptomless controls, which themselves may also have been infected with a still less virulent strain. In Australia, where clear leaf symptoms are rarely seen, Bald (1943) found that the yield of different stocks of Up-to-Date was correlated with the virulence of virus *X* strains they contained, and he estimated that the least virulent strains were reducing yield by 12% and the most virulent by 45%. We have also found differences in yield and vigour of different commercial stocks of Majestic to be correlated with the virulence of the virus *X* in them as indicated by the reactions of inoculated tobacco and *D. stramonium* seedlings. Many of the lower categories of seed potatoes are infected with moderately virulent strains. These often produce no unequivocal leaf symptoms in the field, but if the stocks are compared with virus-free stocks or those infected with avirulent strains, differences in leaf colour and size are usually apparent. There is little doubt that differences in the cropping power of different stocks of one potato variety, when they contain similar numbers of plants with leaf roll and rugose mosaic, usually occur because they contain strains of virus *X* of different virulence. We have encountered stocks of Stock Seed Majestic yielding 40% more weight of tubers than 'H' stocks, a difference that clearly could not be accounted for by the 3% of plants in the latter stocks with leaf roll and rugose mosaic. In deciding on which category of seed to obtain most growers are influenced by the figures for the two aphis-transmitted virus diseases, and pay little attention to mild mosaic, but it is probable, at least in the first year of growing for ware, that the yield will depend more on the type of virus *X* present in the stock than on whether there are 0·1 or 3% of plants with leaf roll and rugose mosaic.

The effects of virulent strains of virus *X* on yield are obvious, and there is no doubt that the general introduction of virus-free potato stocks would increase yields considerably, but it is difficult to get unequivocal evidence about the effects of avirulent strains. The few data there are suggest that they cause appreciable loss. Smith & Markham (1945) inoculated several virus-free varieties in the field when 6 in. high with 'a very mild strain of *X* derived from some Stock Seed Arran Peak potatoes'; infection produced no leaf symptoms, but the yield of the inoculated plants was 12% less than that of the controls. Bald (1944) has reported that virus-free stocks of Up-to-Date in Australia have yielded from 14 to 27% more than 'best certified stocks'. He states that in one trial, 'where there was evidence of an effect of the source and condition of the seed tubers on yield', the virus-free stock yielded less than a local *X*-infected, certified stock.

We have attempted to determine the effects of several strains of virus *X* on the yield of Majestic and Arran Banner. The virus-free stocks used were originally supplied by Dr Kenneth Smith from the Cambridge Plant Virus Station, and had been maintained for many years under glass. In 1944 pot plants in the glasshouse were infected separately with the different strains, while other plants were kept as healthy controls. Majestic plants were infected with an avirulent strain (X^M)

derived from a symptomless plant of Stock Seed Majestic; potato virus *B*, which is either carried or causes a faint mosaic; and a third strain (X^Y) which causes a definite interveinal mosaic. In addition to these three strains, some Arran Banner plants were infected with a fourth strain (X^N), which causes a mosaic in Arran Banner and top necrosis in Majestic. In 1945 the progeny from the glasshouse plants were planted on the Rothamsted farm to multiply sufficiently to use in a yield trial in 1946. The plants were grown in a crop of Stock Seed King Edward well isolated from other potatoes and on land free from volunteer potatoes. Each lot of Majestic and Arran Banner was separated from any other by at least two rows of King Edward plants, and, in addition to the different lots from the glasshouse, one plot of Stock Seed Majestic was also planted. The last was larger seed than the others, and grew much more vigorously early in the season. The Stock Seed and the Majestic with X^M showed no leaf symptoms, but inoculations to *D. stramonium* proved that they were infected. Majestic with virus *B* showed a faint mottle early in the season, but later looked normal, and the X^Y plants were brightly mottled at first but the mottle became less intense later. Arran Banner with X^M and virus *B* showed no clear leaf symptoms, but those with X^Y and X^N both showed a definite mosaic. The tubers were lifted in September and all were kept through the winter in the same store to minimize any effects from differential sprouting or other storage condition.

In 1946 sixty tubers of approximately the same size were selected from each lot and used as 'seed' in a small-scale yield trial. The trial consisted of twelve blocks, six for each variety, each block being surrounded by a guard row of Stock Seed King Edward. The blocks were made up of five rows of ten plants, each row being a different treatment and arranged at random in each block. None of the plants showed any sign of having contracted leaf roll or rugose mosaic during their period in the field in 1945, and tests from the healthy plants to *D. stramonium* showed that they were still free from virus *X*. The tubers were lifted in the first week of October, 3 weeks after the haulms had been killed by *Phytopthora infestans*, and the produce from each row was pooled and weighed. The results are given in Table 1 and show that infection of the virus-free stocks with any of the virus strains used reduced the yield. Unfortunately, the land used for the trial was very variable and there were large differences between the yields of different blocks, leading to a high standard error, but the reductions caused by virus *B* and X^Y in Majestic and X^N in Arran Banner are statistically significant.

Perhaps the most interesting result of the yield trial is that the Stock Seed Majestic, in spite of being infected with an avirulent strain of virus *X*, yielded better than the virus-free plants, though the increase is not statistically significant. One explanation of this may be that the virus-free stocks were suffering from the effects of continued propagation under glass, and that longer than one year in the field may be needed to mitigate this condition. However, it is equally possible that there are differences of vigour and cropping power within potato varieties. Werner (1940) has described

such differences in clonal strains of Triumph potatoes, though he does not seem to have eliminated the possibility of the differences being caused by infection with different strains of virus *X*. There is no doubt that most of the variations in cropping power of different stocks of one potato variety are determined by viruses, but it would be unjustifiable to assume that there are no other causes, and that a virus-free plant of one variety must of necessity crop better than one that is virus infected. Variations within potato varieties that affect foliage type and habit of growth, for example, bolters and wildings, are common. They presumably arise because of somatic mutations, and there is no reason to believe that mutations may not also occur that affect cropping power without producing any change in foliage characters. We have examined clonal lines of the variety May Queen, supplied by

TABLE 1. *The effect of different strains of virus X on the yield of Majestic and Arran Banner potatoes*

	Majestic		Arran Banner	
	Average yield per plot (10 plants)	Yield as percentage of virus-free seed	Average yield per plot (10 plants)	Yield as percentage of virus-free seed
Scotch Stock Seed	14·2 kg.	110	—	—
Virus-free	12·9	100	14·2 kg.	100
X^M-infected	12·3	95·3	12·8	90·2
X^B-infected	10·5	81·4	13·2	93·0
X^Y-infected	9·8	76·0	12·7	89·4
X^N-infected	—	—	11·1	78·2
Standard error	±0·6	—	±0·7	—

Mr A. Beaumont, which varied in height, vigour and maturity, but could detect no virus in any by inoculation tests to tobacco and *Datura stramonium* or by grafting to other potato varieties. Thus, in searching for new clonal lines to replace the existing commercial potato stocks, freedom from virus *X*, although a primary consideration, should not be the only criterion, and before such lines are widely developed they should be shown to be at least as desirable in respect of other agronomic characters as the best stocks now in cultivation.

THE SPREAD OF VIRUS *X*

The selection and propagation of desirable clones of potatoes free from virus *X* will be worth doing only if they can be maintained in this condition without too great difficulty. Whether this is possible can be decided with certainty only as a result of experience, but on our present knowledge the attempt would seem well worth making and likely to succeed. It is true that some infections of potatoes with virus *X* lack an explanation, and there may be serious gaps in our knowledge of the methods whereby it can be transmitted. Nevertheless, there is little doubt that most infections occur because of mechanical transmission of the virus, either as a result of contact between healthy and infected living plants or between healthy plants and

material contaminated with the virus. There is no evidence to suggest that the virus is insect-transmitted. Many workers have tried numerous insects without success, and we have made repeated tests of potato aphides and other leaf-feeding insects under a variety of different conditions but have consistently failed to get transmission. Nor does the manner in which the virus spreads in the field suggest transmission by insects, for we have never found healthy plants to become infected unless they were in contact with sources of infection. It is impossible to prove a negative, but it can be concluded that if transmission by insects does occur it must be rare, though the spread that occurs between plants in contact below ground (Roberts, 1946) could be caused by soil insects or other fauna.

Experimental data on the rate at which virus *X* spreads are few, but they all suggest that spread is slow and that once virus-free stocks have been produced in quantity they will remain healthy for long periods provided a few elementary precautions are taken. There are some facts that are often adduced as evidence that the virus spreads easily and rapidly and that virus-free stocks would soon become as badly infected as those now in commerce. For example, the virus occurs annually in some tomato crops and is widespread in some relatively new potato varieties, such as Arran Peak. The exact interpretation of such facts, however, is uncertain. First, it is doubtful if what happens in tomato crops is relevant to the potato crop. The tomato crop is continually being handled by workers and chances of introducing the virus are high, and Roberts (1946) has shown that much more spread occurs between tomato than between potato plants. Also, our results show that tomato plants under glass are much more susceptible to infection, that is, infection is caused by inoculum containing less virus, than are potato plants, and a higher concentration of virus is reached in infected tomato plants. Hence introductions of virus *X* from outside sources, which are far from common in tomato crops, can be expected to be still less frequent in potato crops, and the spread from any such initial sources of infection will be much slower. Secondly, the prevalence of virus *X* in some recently introduced potato varieties is far from unequivocal evidence of repeated new infections or of rapid spread in the variety. Infection may well have occurred early in the life of the variety, when it consisted of only one or a few plants, and the present distribution may be simply the result of the subsequent propagation of tubers from these plants. There has been plenty of opportunity for much early infections, because potato breeders have taken few precautions to isolate their new seedlings from contact with infected stocks. It would save much trouble in the future if, in the first years of a seedling's existence, more attention were paid by potato breeders to the control of virus *X*, by planting their new products on land free from volunteer potatoes and well out of contact with other infected plants.

The few experiments on the rate at which virus *X* spreads in potato crops have been made in such different conditions that it is difficult to draw any general conclusions, but the rate of spread seems to vary with different varieties and with different strains of the virus. Schultz, Clark, Raleigh, Stevenson, Bonde & Beaumont (1937) found that Katahdin plants remained uninfected when grown

alternating with infected plants, whereas in the same conditions 70% of the plants of a Green Mountain seedling became infected when interplanted with Triumph with 'latent mosaic' and 33% of those interplanted with Green Mountain with 'latent mosaic'. In one experiment in which Loughnane & Murphy (1938) alternated healthy and *X*-infected potatoes of unspecified varieties at a distance of 10 in. apart eleven out of twenty-four healthy plants contracted the virus, whereas in a second experiment with President, Champion and Arran Banner, only two out of fourteen plants alternating at a foot distance with infected plants contracted the virus. Roberts (1948), working with individual infectors surrounded by healthy plants, found on an average that less than 1 in 10 of the healthy plants adjacent to infected ones contracted the virus in one season, but in similar conditions more infections occurred with Majestic than with Arran Banner and some strains spread more freely than others. Thus, increase in the proportion of infected plants in a stock will be slow, the available data suggesting annual increments of between one-tenth and one-third of the number of infected plants already present. It seems that there is little chance of virus-free stocks suddenly 'breaking-down', but rather that it will be a period of years before stocks in which a few plants have become infected from outside sources contain a significant proportion of infected tubers. With less than 10% of the stock infected, loss from virus *X* will be unimportant, and, on existing evidence, the increase from 1 to 10% will take from 3 to 7 years, during which time it should become apparent that the virus has entered.

There is no experimental evidence of potatoes becoming infected with virus *X* under conditions in which contact with a source of the virus could be excluded with certainty. It seems, therefore, that virus-free stocks should remain in the condition as long as they can be isolated from other potatoes. This is borne out by the fact that Clinch, Loughnane & Murphy (1938) maintained several lots of Champion free from virus *X* in Donegal under commercial conditions for 6 years. However it must be stressed that the virus is readily transmitted mechanically and is fairly stable, so that it may occasionally get introduced into healthy stocks on workers' hands, implements or even on the beaks of birds, such as crows, that eat tubers. Such introduction will be rare and cause infections of only isolated plants, and there is little doubt that the greatest danger to the virus-free stocks will come from infected volunteer potatoes surviving from previous crops. Doncaster & Gregory (1947) have found that in the year following a potato crop, volunteers may amount to several thousands of plants to the acre, and that even 4 years later with some rotations there may be several hundreds. Volunteers come up within the crop and are ideally placed as sources of infection, and to avoid this mixing with infected plants the virus-free stocks should be propagated and maintained on land free from other potatoes.

Compared with the danger of infection from volunteer potatoes other sources of infection for virus-free stocks are probably of little importance. Once the virus has entered a stock, however, it can spread during storage as well as during the growing season. It has often been suggested that transmission from infected to healthy tubers

might occur during cutting, but our results suggest that this is uncommon. We have cut fifty virus-free tubers into four or more pieces each, using for each cut a knife that had just previously been used for cutting infected tubers, but all the pieces gave virus-free plants. It seems likely that the only danger of transmitting virus *X* in this way would be if the knife were to cut first a sprout on an infected tuber and then one on a healthy tuber. The storage parenchyma has too low a virus content to be a dangerous source of inoculum and also seems to be resistant to infection by inoculation. We have rubbed freshly cut surfaces of sixteen Doon Star and Arran Banner tubers with expressed sap from infected tobacco plants, but when planted all the inoculated pieces produced healthy plants. Sprouts, on the other hand, became infected when they were rubbed with such inoculum, but different results were obtained with the two varieties. Of 10 Doon Star tubers on which one sprout each was lightly rubbed with infective sap, seven gave infected plants, but six Arran Banner tubers treated in the same manner remained uninfected.

McIntosh (1944) has suggested that virus *X* may spread as a result of contact between sprouts of healthy and infected tubers during storage, and described one infection out of ten plants where sprouts were rubbed gently with those of infected tubers. Our results show that a high virus content is achieved in developing sprouts and that sprouts are also susceptible to infection, so that such a method of spread is clearly one that might be important, especially if potatoes are roughly handled when sprouting. This conclusion was confirmed by an experiment in which 100 virus-free Majestic tubers were marked and placed in a sack with fifty *X*-infected tubers. At approximately fortnightly intervals from the beginning of January to the middle of April the sack was shaken. The tubers were then planted in the field out of contact with other potatoes, and inoculation tests in June from the leaves to *D. stramonium* showed that fifteen of the plants from previously healthy tubers had contracted virus *X*.

In maintaining stocks of virus-free potato varieties, therefore, they should be kept from contact with other stocks throughout the year, when it seems probable that they will remain virus-free for long periods. Provided they are propagated in the cool, moist climates now used as potato-seed growing areas, it is likely that if sporadic infections occur most of these will be identified by field inspections. Along with field inspections, however, should go tests for virus *X* to ensure that the stocks do not contain significant numbers of plants infected with strains that cause no leaf symptoms. Such tests need not be elaborate and can as well be done on the sprouted tubers as on the growing crop. They can be made either by inoculation tests or by serological methods, and they need not aim at detecting infection until it exceeds 1% of the stock. Sap from both the foliage and the developing sprouts contains enough virus to give infection on *D. stramonium*, or a precipitin reaction, when diluted ten times with sap from healthy plants. Hence pooled samples from ten plants can be tested together, and ten such tests on a stock would probably be enough to detect the virus before it becomes distributed widely enough to be of any economic importance.

BAWDEN and OTHERS—*Studies on the importance and control of potato virus X*

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EXPLANATION OF PLATE 5

Fig. 1. Majestic plant from Stock Seed infected with a virulent strain of virus *X*, causing severe necrosis and dropping of the lower leaves.

Fig. 2. Plant protection test. Leaves of same age from two *Datura stramonium* plants photographed 8 days after inoculation with a virulent strain of virus *X*. The right-hand leaf was from a healthy plant and shows severe necrosis. The left-hand leaf was from a plant previously inoculated with sap from a healthy-looking Majestic potato; it is infected with an avirulent strain, which produces only an indistinct mottle, but which protects it against the virulent strain.

Fig. 3. The separation of strains of virus *X* in one plant. Comparable leaves from two shoots of one Majestic plant from a Stock Seed field crop. The left-hand leaf shows no clear symptoms and contains an avirulent strain; the right-hand leaf is severely necrotic and crippled and contains a virulent strain.

(Photographs by V. Stansfield)

(*Received* 31 *July* 1947)

EXPERIMENTS ON THE SPREAD OF POTATO VIRUS *X* BETWEEN PLANTS IN CONTACT

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Experiments on the spread of five strains of potato virus *X* were made with seven potato varieties and with tomato plants both under glass and in the field. Spread by leaf contact between healthy and infected plants was confirmed, and it was also found that spread could occur between plants whose only contact was below ground.

The rate of spread was much greater in tomato than in potato plants, and virulent strains of the virus, which achieve a high concentration in infected plants, spread more rapidly than avirulent strains. In only one experiment with potatoes did more than 10% of the healthy potato plants exposed to infection become infected during one season.

Datura stramonium and tomato plants became infected when growing in soil containing sap or residues from *X*-infected plants.

It was common in the field for potato plants whose foliage gave no reaction for virus *X* at the end of the season to yield a mixed progeny of healthy and infected tubers. Such infections are thought to result from underground spread.

Attempts to transmit virus *X* from infected to healthy potatoes by means of *Rhizoctonia solani* failed. No examples of infection were found except when healthy plants came into direct contact with sources of the virus.

Introduction

Loughnane & Murphy (1938*b*) showed that potato virus *X* could spread from plant to plant by leaf contact in conditions which exclude insect participation. Work at Rothamsted during the last three years has confirmed this method of spread and shown that in tomatoes (Roberts, 1946) spread from plant to plant also occurs underground. The exact mechanism of this underground spread is unknown. There are indications that it also occurs with potatoes, and that it may prove to be responsible for at least as many new infections as is leaf contact.

Methods

Tests for virus *X* were made by inoculation to seedlings of tobacco (var. White Burley) and *Datura stramonium* with checks on doubtful plants being made by serological methods or plant protection tests as described by Bawden, Kassanis & Roberts (1948). For tests on tubers, inoculum was taken from sprouts when possible, otherwise a cone-shaped piece of the tuber bearing an 'eye' was used. In testing potato haulms, care was taken to ensure that leaves from every stem were included in the sample crushed to provide inoculum.

To ensure the use of virus-free potatoes in the experiments, the tubers were all tested shortly before they were to be used and tests were repeated as soon as foliage came above ground; as a further check on the health of the tubers used in some of

the field experiments 'eyes' were cut from the tubers and were planted for observation in an insect-proof glasshouse. The virus-free potatoes used in the experiments were all derived from tubers originally supplied by Dr Kenneth M. Smith, Plant Virus Station, Cambridge.

The strains and source of virus used in the varietal trials were as follows:

X^M. Mild strain isolated from symptomless Majestic Stock Seed.

X^B. Potato virus *B* obtained in pure form from the variety Duke of York (Cockerham, 1943; Bawden & Sheffield, 1944).

X^Y. Strain found in naturally infected Majestic in the field which causes a yellow mottle reaction in Majestic, *D. stramonium*, tobacco and tomato.

X^N. Strain which causes mosaic and necrosis on President and Arran Banner, top necrosis in Majestic, and a ringspot reaction in tobacco and *D. stramonium*.

X^K. Strain isolated from one shoot of a Stock Seed Majestic plant which showed a conspicuous mosaic and necrotic spots. It causes stunting and reduction in leaf size with a mottle and necrotic spotting on five of the varieties used. In King Edward plants it causes top necrosis, and in tobacco a severe ringspot.

Experimental data

1944. *Spread of potato virus X experiment; vars. Majestic and Arran Banner*

This experiment and the succeeding one were laid out according to a plan agreed upon by the Potato Diseases Committee of the Agricultural Research Council. In addition to those at Rothamsted which are described here, identical field experiments were made by Dr G. Cockerham at Edinburgh, Dr Kenneth Smith at Cambridge, and Dr T. Whitehead at Bangor.

The virus-free Majestic and Arran Banner tubers were supplied to the four centres by Dr Kenneth Smith, and the infected Arran Banner and Kerr's Pink tubers were obtained through Dr Whitehead from ordinary infected commercial stocks. A preliminary test was made from these tubers by inoculation to tobacco and *D. stramonium* to confirm that they were respectively healthy and infected.

Plan of experiment. The tubers were planted early in April according to the agreed plan, with two plots, one containing infected plants and one control, of each variety. Plants were spaced at $1\frac{1}{2}$ ft. in rows 2 ft. apart. The Majestic plots consisted of four rows of fifteen plants each, but in the infected plot, *X*-infected Arran Banner tubers replaced Majestic in the 3rd, 8th and 13th position. The Arran Banner plots consisted of only two rows each, with Kerr's Pink tubers as infectors. The Majestic and Arran Banner tubers were all cut, and halves from the same tubers were planted in the corresponding positions in the two plots. To fill the positions in the control plot corresponding to those occupied by the infectors in the other, tubers occupying adjacent positions were further subdivided. The plots were surrounded by guard rows of King Edward Stock Seed.

Tests on foliage. Tests from the foliage of the Majestic plots were made on

D. stramonium in late June, mid-August, and mid-September. No positive reactions for *X* were obtained from any of the previously healthy plants. Leaves from the Arran Banner plots were tested in early June, when none reacted positively, and again in late August, when one plant from the infector plot gave a positive reaction. This plant was next to an infector.

Tuber tests. Tubers were lifted at the end of September, and three were kept from each plant. Some rotted in storage, but the survivors were tested in the following January by inoculation to tobacco plants. No test plant reacted positively from any tuber in either of the control plots, but tubers from seven plants in the Majestic plot, and five in the Arran Banner were infected with *X*. The latter included one out of two tubers tested from the one Arran Banner plant which had previously given a positive reaction in the foliage test.

All of these infected tubers came from plants which had been growing next to infectors, and as the majority of the tests showed that not all the tubers from one plant were infected, it seemed that a mixture of infected and healthy tubers from the same plants is a common phenomenon. It was also apparent that tests made from the foliage, even at the end of the season, gave no reliable indication of the health of the tubers.

1945. *Spread of potato virus X experiment; var. Gladstone*

In 1945 a similar experiment was carried out with the variety Gladstone. Planting was done early in May in two plots (infector and control) which each consisted of two rows of fifteen plants. Individual tubers were numbered and halved, and one-half of each was planted in corresponding positions in each plot. In the infector plot, infected tubers replaced the Gladstones in the positions 3 (X^M, Majestic), 8 (X^B, Duke of York), and 13 (X^Y, Katahdin) in each of the two rows. In spite of late planting, growth was good and large haulms were produced. All the tubers harvested from each plant were kept for testing.

Field tests on foliage. Early in June leaves from each plant in the infector plot were tested before they came into contact with one another. None of the Gladstone plants reacted, but all the infectors did. All the plants were again tested in the first week in September with the same results as in June. Plants in the control plot were tested early in August and again in September, with negative results.

Tuber tests. A selection of four tubers which included the largest, the smallest, and two intermediate in size, was made from the progeny of each plant. Each tuber was tested individually by inoculation to a tobacco and *D. stramonium* seedling. Tubers from the infector plants were also tested, and these all gave positive reactions.

The only positive reaction from all the Gladstones was obtained with one tuber, the smallest of the four tested, from a plant which had grown next to one of the X^Y infectors. All eighteen tubers from this plant were then tested and tubers numbered 2, 3, 4, 6, 13 and 18, ranging from the second largest to the smallest, reacted positively. The rest gave no reaction and were presumably virus-free, confirming

the observation made on the 1944 experiment that mixed progenies can arise from current year infections with potato virus *X*.

A feature of these results is that the only infection of a previously healthy plant exposed to virus *X* was revealed by tuber tests, as were all but one of the infections which occurred in the experiment of the previous year. Again it was apparent that foliage tests at the end of the growing season are not a reliable indication of absence of current season infection, and that if there had been exposure to chance of infection, virus *X* may be in some tubers set by a plant but not in others.

Spread of different strains of virus X in different varieties of potato

In each of the years 1944–6, small-scale field trials were made to compare the rate of spread of four different strains of the virus through different varieties of potato.

1944 *field trial.* The varieties used were Arran Banner, Arran Pilot, Doon Star, Epicure, Kerr's Pink, King Edward and Majestic. With the exception of the King Edward these were all tested before planting to confirm initial freedom from potato virus *X*. In addition, some Golden Wonder tubers were included in the trial, but these were planted in a row with an *X*-infected Kerr's Pink in every alternate space. None of these plants showed obvious symptoms of infection during the growing season, but tests made from the foliage a few days before they were harvested showed that three out of the ten plants exposed had been infected.

Each lot of varieties was planted with one of the strains of *X* as the infector in plots isolated from one another by guard rows of Stock Seed King Edward. A plot consisted of four tubers of each of the seven varieties planted two on either side of an infector tuber. With the exception of the X^M plot, in which tubers were set 12 in. apart, the spacing was 18 in. between plants, and 2 ft. between rows.

A test was made from the foliage of every plant in the four plots shortly before harvesting, and all the tubers were kept for testing later. From all the plants (i.e. 112, comprising sixteen of each variety) only one positive reaction for *X* was obtained. This was from a Majestic plant which had grown next to a plant infected with X^M. Tuber tests were made on five tubers from each plant, and these showed that again more spread had occurred than had been revealed by the foliage tests, as tubers from four plants in all were infected. All the tubers set by the Majestic plant whose leaves had reacted positively were infected, but three other plants in the X^M plot also reacted. The infections were in a Doon Star plant, growing next to an infector, of which all five tested tubers were infected, and in a Majestic and an Arran Banner plant growing one plant removed from the infectors. With each of these only one of the five tubers tested was infected. At the spacing in this plot, 12 in., there was ample opportunity for foliage or root contact between these plants and the infectors.

Tuber tests failed to reveal any infections among the plants in plots infected with the other three strains of *X*. Thus the total number of infections out of 112 plants exposed to various strains of *X* in this trial was four only. On the basis of the tuber

tests, twelve out of the 560 tubers, or 2%, were infected. The results of this, and the trials of the two following years, are shown in Table 1.

During the three years in which these field experiments have been made, Stock Seed King Edward has been included among the varieties tested, and in each year one, and in 1946 two instances of infection by virus *X* were noticed. The King Edward tubers were not tested before planting, so it is uncertain whether the virus was present in the Stock Seed, or was acquired by contact with the infector plants. These infections are interesting, especially as no King Edward plants were ever seen to exhibit top necrosis in the field although in 1946 the foliage of one plant reacted positively on testing. The other three occurrences of infection by virus *X* in this variety were revealed by tests from tubers. The *X* reaction obtained on tobacco in 1946 from a tuber of a plant growing next to an X^K infector was of the severe type characteristic of the infector. One of the non-reacting tubers from this King Edward plant and one from the Doon Star infector were grown in separate pots in the glasshouse, and sap from the young shoots of the Doon Star plant was used as inoculum for the others. Only a local reaction was obtained; the two plants were allowed to mature with their foliage in contact, after which leaves from the top of the King Edward plant, and the three tubers which had developed were tested individually on tobacco with one tuber from the Doon Star plant. The latter was infected, but neither the leaves nor the tubers from the King Edward plant gave a positive reaction, indicating a possibility that underground contact may be essential for the transmission of virus *X* to this variety.

1945 *field trial.* In 1945 the varieties Arran Banner, Doon Star, Gladstone, Kerr's Pink, King Edward and Majestic were used with the same strains of *X* as in the previous year, except that the strain X^K was substituted for X^N. The experiment was planted in the same way as in the previous year except that there were two plots of each lot of varieties for each of the four strains and all the plants were set 18 in. apart.

Tests were made from the foliage at the end of the season, and three tubers from each plant were tested, one, the largest, as an individual test, and the remaining two as a pooled test. Table 1 shows that more spread occurred than in the previous year, and that the severe strain X^K accounted for the greater part of the increase. Out of 192 plants exposed, seventeen (including one King Edward) became infected. More than half of these were revealed by tests from the tubers, and not from the leaves. The variety in which the greatest number of infections (six) occurred was Majestic, but these were concentrated in one block of plots; in the second block there were no infections in this variety.

1946 *field trial.* In this trial the same six varieties were used with the same strains of *X* as in 1945. Again the plots were laid out in two blocks, so that each variety was exposed twice to each strain of *X*.

Tests made from the foliage at the end of the season again revealed only a few of the total number of infections. Out of the 192 plants exposed, thirty-one, including

TABLE 1. *Spread of some strains of virus X in different potato varieties in* 1944, 1945 *and* 1946

No. of plants exposed to infection	Approx. no. of tubers tested	Variety							Strain of virus *X*	Block	Year
		Arran Banner	Doon Star	Kerr's Pink	King Edward	Majestic	Arran Pilot	Epicure			
112	560	*A_4 (T)	D_2 T	—	KE_1 (T)	M_2 (L, T), M_4 (T)	—	—	*M*	—	1944
		—	—	—	—	—	—	—	*B*		
		—	—	—	—	—	—	—	*Y*		
		—	—	—	—	—	—	—	*N*		
192	576	Arran Banner	Doon Star	Kerr's Pink	King Edward	Majestic	Gladstone	—			1945
		—	—	—	—	M_3 (L, T)	—	—	*M*	I	
		—	—	K_3 (T)	—	M_2 (T), M_3 (T)	G_2 (T)	—	*B*		
		—	—	—	—	—	—	—	*Y*		
		—	D_3 (L), D_1 (T)	—	KE_4 (T)	M_1 (L), M_2 (L, T), M_3 (L)	G_4 (L)		*K*		
		—	—	K_3 (T)	—	—	G_3 (L)	—	*M*	II	
		—	—	—	—	—	—	—	*B*		
		—	D_2 (T), D_3 (T)	—	—	—	—	—	*Y*		
		—	D_3 (T)	—	—	—	—	—	*K*		
192	576	Arran Banner	Doon Star	Kerr's Pink	King Edward	Majestic	Gladstone	—			1946
		—	D_2 (T)	K_2 (T)	—	—	—	—	*M*	I	
		—	D_3 (L)	—	—	—	—	—	*B*		
		A_3 (L, T), A_4 (L,T)	—	K_2 (L, T), K_3 (T), K_4 (L)	KE_3 (L)	M_3 (L), M_2 (T)	G_4 (T)	—	*Y*		
		A_3 (T)	—	K_3 (L, T), K_2 (T)	—	M_2 (L, T)	—	—	*K*		
		—	—	K_2 (T)	—	—	—	—	*M*	II	
		A_3 (T)	D_2 (L, T)	—	—	M_2 (L, T) M_3 (T)	—	—	*B*		
		A_1 (T), A_2 (T)	D_3 (T), D_4 (T)	K_3 (L, T), K_4 (T)	—	M_3 (L, T), M_2 (T)	—	—	*Y*		
		—	—	K_2 (L, T)	KE_3 (T)	—	—	—	*K*		

* Numeral after the initial letter of variety indicates position of plant in relation to infector, i.e. nos. 2 and 3 are plants on either side of infector. (L) indicates positive reaction from leaf tests, (T) positive reaction from tuber test.

two King Edward plants, became infected, a considerable increase over the results of the previous year. Of the thirty-one infections, only eight occurred among the plants exposed to the mild X^M and X^B strains, while the severe X^Y strain accounted for seventeen of the remaining twenty-three infections. Distribution of infection among the different varieties in each of the three year's trials can be seen in Table 1.

Glasshouse experiments on spread of X in potato plants

The first controlled experiments to test the spread of virus X among potatoes in the glasshouse were made by Loughnane & Murphy (1938*a*), who observed that under the conditions of their experiments about twice as many plants in contact with infected plants contracted the virus in the glasshouse as in the field. The results of some of the experiments reported here bear out this observation, although in other tests few or no plants contracted infection by contact.

Exp. I. *Spread of X from Majestic to Doon Star*

In this test Doon Star plants were used as the healthy contacts and Majestic infected with X^Y as infectors. Thirty of the Doon Star tubers were halved. One lot was planted in 8 in. pots arranged in groups of five about a central pot containing an infected Majestic tuber. The control consisted of the other lot of halves similarly arranged on an adjoining bench, but about a central healthy plant. When the healthy plants were 3–4 in. high, and before there had been any contact with the infectors, leaves from each were tested for virus X and none reacted. The Majestic plants showed a conspicuous mottle. Contact between healthy and infected plants took place at about the end of March and continued until June. Maximum opportunity for contact between diseased and healthy foliage was assured by staking the individual groups of plants in such a way that the foliage of the one diseased plant in the centre and of the five healthy surrounding plants was thoroughly mingled. Seven of the thirty contact plants showed symptoms, and when all the plants were tested again on *D. stramonium* these were the only ones which gave a positive reaction for X. Infections were not evenly distributed through the six groups, but were made up by three plants in each of two groups and one in another. Plants in the remaining three groups escaped infection. None of the controls became infected; they showed no symptoms, and their freedom from virus was later checked by inoculating them with X^Y from tomato.

Exp. II. *Spread of three strains of virus X to Doon Star and Arran Banner from infected plants of the same variety*

This test, somewhat similar to the preceding one, was made with the varieties Doon Star and Arran Banner, and three strains of X—X^M, X^B and X^N. Infectors were provided by inoculating healthy young plants of the two varieties with the appropriate strain of virus. Plants were arranged on the glasshouse bench in groups of five about an infector as in the last experiment, with two groups of each variety

for each virus strain. The test was started in June, and the final tests on leaves were made at the end of September. The results, shown in Table 2, agree with those of the last experiment in suggesting that spread of *X* by foliage contact is not rapid even under glasshouse conditions.

Table 2

	Strain of *X* virus			Total no. infections out of	
	X^B	X^N	X^M	30 plants	60 plants
Doon Star Group 1	0	3	1	6	—
2	0	1	1		
Arran Banner Group 1	1	1	1	3	9
2	0	0	0		
Total no. infections per group	1	5	3		

Exp. III. *Spread of virus X from Majestic to Kerr's Pink, with root and foliage and root contact only*

This experiment was designed as an additional test of glasshouse spread of virus and also in connexion with some tests which were being made in an attempt to transmit virus *X* from infected to healthy tubers by means of *Rhizoctonia solani* which will be described elsewhere.

Eighteen 12 in. earthenware crocks, filled with sterilized soil, were divided into three lots with one infected Majestic and one healthy Kerr's Pink tuber planted on opposite sides in each. In the first lot of six pots the Majestic tubers had previously been inoculated with *R. solani*, and in addition the soil round the tubers was heavily contaminated with agar cultures of the fungus. In the 2nd and 3rd lots, the Majestic and Kerr's Pink tubers were surface sterilized with 1/1000 HCl before planting. As soon as shoots appeared above ground, screens of double cellophane were erected so as to prevent any contact between infected and healthy plants in all the pots of lots 1 and 2. The pots in lot 3 were unscreened, and free contact took place between the foliage, and presumably the roots, of the Majestic and Kerr's Pink plants. The test was started at the beginning of April, and was concluded when the haulms began to mature.

Foliage tests were made shortly before the tops died; these were all negative, i.e. none of the healthy Kerr's Pink plants grown in the same pots as infected Majestic plants gave a positive reaction either in the twelve pots with screens, or in the six which were unscreened.

Tests were made later on sprouts from the tuber progenies of these plants. One tuber from each Majestic plant was tested, and all gave positive reactions for *X*. Two or three tubers from each of the Kerr's Pink plants were also tested and the only positive reaction was from one of the two tubers tested from a plant which had grown in one of the screened pots of group 2 in which the only contact with the infector had been underground.

Exp. IV. *Spread of virus X from Majestic to Gladstone, with root and foliage, and root contact only*

A test similar to the above was made in 1945 using healthy Gladstone tubers and Majestic tubers infected with X^{M}, one of each kind being planted in each of eighteen 12 in. pots. The shoots of the Gladstone plants were tested to confirm their freedom from *X* as soon as they appeared above ground. Cellophane screens similar to those used in the preceding experiment were placed in nine of the pots between the infected and healthy plants, and the plants were staked so as to prevent healthy plants in screened pots from coming into contact with any source of infection. This test was started in May, and final tests on the leaves were made in September, after which the tubers were harvested and stored.

Foliage tests showed one positive reaction for *X* from one of the Gladstone plants grown in an unscreened pot, where there had been full contact with the foliage of the infected Majestic. The other half of the tuber from which this plant originated had been planted in a screened pot, and its foliage reacted negatively.

In the following January one tuber from each of the infected Majestic progenies, and four from each of the Gladstone plants were tested. All the Majestic tubers gave positive reactions for *X*, as did all four of the Gladstone tubers from the plant whose foliage had also reacted positively.

Spread of potato virus X in glasshouse-grown tomato plants

Experiments carried out with tomatoes in the glasshouse have shown that, contrary to experience with potatoes, spread of virus *X* from infected to healthy plants by leaf contact occurs in this host with a facility comparable almost with that of tobacco mosaic virus. The methods used were similar to those described with potatoes, that is, plants were grown in 8 in. pots and 'infector' plants were inoculated with the appropriate strain of *X* when they were 3–6 in. high. Five healthy plants were then grouped around each infected plant in such a way that, as they grew, leaves from all of them could come into contact with the central plant. Once an experiment was set up, and the central plant inoculated, the position of the pots was not altered, and disturbance of the leaves and stems was reduced to the minimum necessary for staking. Leaves from all 'contact' plants were tested on tobacco and *Datura stramonium* before discarding an experiment.

Spread of four different strains of virus X in tomato plants with leaf contact only

In one experiment twenty-five healthy tomato plants were exposed to infection by X^{Y} virus, and tests of the foliage showed that fourteen of these, i.e. 56% had become infected. A similar test was made using the three strains X^{B}, X^{N} and X^{K} as inocula for the infector plants. Reactions given by the 'contact' plants on tobacco and *D. stramonium* at the end of the experiments showed that 19/29, 8/14 and 8/25 plants had become infected, or 65·5, 57·0 and 32% for the three virus strains respectively. The fewer infections among the 'contact' plants from the severe X^{K}

strain may have been due to the drastic reaction and stunting of the infectors; plants reacted to inoculation by the production of dark, necrotic, interveinal lesions, the inoculated leaves died early, while the remainder of the plants became stunted, light green, and generally mottled.

Spread of X^Y in tomato plants with root and leaf, and root contact only

Exp. I. These experiments, part of which have already been briefly described (Roberts, 1946), were similar in plan to that carried out with Gladstone and Majestic potatoes. Eighteen large earthenware pots, filled with sterilized soil, were each planted towards the end of March with two 3–4 in. high tomatoes at opposite sides. In nine of the pots screens of cellophane were placed between the two plants, and a few days after transplanting one plant in each pot was inoculated with X^Y. Within 10 days, the inoculated plants gave clear symptoms of systemic infection. Liquid dressings containing nitrogen, phosphorus and potassium were applied twice during the experiment, and the leaves of all the uninoculated plants were tested during the 1st week of June by inoculation to tobacco.

Seven out of nine plants in the unscreened pots in which there had been full contact with infected plants reacted positively; in the pots where foliage contact between infectors and 'contact' plants had been prevented by means of screens, five out of nine plants reacted positively.

Exp. II. The screens in the individual pots were believed to have been adequate to prevent any leaf contact, but in a second experiment a still more effective type of screen was used to separate the healthy from the infected plants. In the first week of July eighteen pots were each again planted with two young tomatoes, and nine of these were placed in a row on a glasshouse bench. A continuous barrier of double cellophane was run between the two plants in each of these pots, and this was extended 1 ft. beyond each end. The barrier was continued $1\frac{1}{2}$ in. below soil level by strips of cellulose acetate. After the screen was in position, one plant in each of the eighteen pots was inoculated with the X^Y strain of virus. All watering of the screened pots had necessarily to be done from the sides which contained the healthy plants, as the other side was not accessible, and, in staking, the plants were trained away from the screen. Symptoms of infection in the inoculated plants were obvious within a fortnight after inoculation.

After approximately 2 months all the uninoculated plants, many of which were showing symptoms of infection with X^Y both in the screened and unscreened pots, were tested on *D. stramonium*. The results showed that all nine plants from the screened pots, where the only contact with infectors had been through the soil, had become infected, and seven out of nine plants from the unscreened pots were also infected.

Exp. III. This test was a continuation of the preceding one. The screen was left in position, and all the 'contact' plants including as much of the root systems as possible were carefully removed from the eighteen pots. Infector plants were left

undisturbed except for top pruning to encourage new lateral growth. Most of the pots were then replanted with healthy young tomatoes early in October, and approximately 6 weeks later these were tested on tobacco when five out of nine plants from the screened pots reacted positively, and seven out of seven from the unscreened.

Infection of tomato plants through infected soil

A preliminary test, which resulted in one positive reaction out of four plants treated, had demonstrated that infection might result from watering the pot soil with virus *X*-infected plant sap. The method used was as follows. 30 c.c. of sap was expressed from tomato plants infected with X^Y. This was diluted approximately 1/4, and 40 c.c. of the solution was poured from the side of the pots over the soil. Previously, any leaves which were near or touching the soil had been removed, and care was taken not to allow any fluid to come into contact with any part of the plant above soil level. Symptoms of infection became clear 2–3 weeks after treatment on one of the plants, and this was confirmed by tests on *Datura*.

A similar test was made on four young plants each of tomato, tobacco, and *D. stramonium* with 50 c.c. of infected sap diluted 1/5. Contact between the lower leaves of the tobacco plants and the soil was prevented by means of strips of stiff white paper, and the lower leaves of the two other plants were removed. The day after the sap had been applied to the soil an attempt was made to damage the roots of two of each of the host plants by driving the blade of a sterilized scalpel into the soil at four points. Five weeks later, all the tomato plants were clearly infected with the virus, but none of the tobacco or *D. stramonium* plants showed any symptoms. The roots and leaves of the latter were tested separately on tobacco after 6 weeks, and again none gave a positive reaction.

A test was then made to see whether plants could become infected with X^Y by being grown in soil which had formerly contained infected plants, and for this purpose soil which had carried X^Y infected tomato plants in the glasshouse continuously for 8 months was used. There were three treatments; the first consisted of soil from which all the large root remains of the old infected plants had been removed by passage through a coarse sieve, and the second of unsieved soil to which additional inoculum was added in the form of chopped-up roots of old infected tomato plants. The third treatment consisted of ordinary sterilized glasshouse soil to each pot of which 25 c.c. of X^Y infected plant sap, diluted 1/6, was added 2 days after the plants used in the test had been transplanted. Each treatment employed thirty plants, ten each of tobacco, tomato and *D. stramonium*, all of which were young seedlings at the beginning of April when the experiment was started.

Within a month of the start of the experiment two of the tomato plants and one *D. stramonium* from treatments 3 and 2 respectively were clearly infected. In June, tests of the roots of all the plants in the three treatments showed that out of thirty plants grown in infected soil to which roots had been added only one, the *D. stramonium* previously noticed, had become infected. No infections occurred in any plant in the sieved infected soil treatment, or among the tobacco or *D. stramonium*

plants in the glasshouse soil to which infected sap had been added, but three out of ten tomato plants in this last treatment were infected. Two of these infections in tomato were from those plants in which symptoms of X^Y had been observed early in the experiment. As a result of the root tests of the tomato plants, which were made on tobacco, these same two plants gave systemic infections in addition to one plant which gave ringspot-type local lesions on the inoculated tobacco leaves. Reinoculation from one of these local lesions on *D. stramonium* resulted in a systemic *X* reaction.

Attempts to transmit potato virus X by means of Rhizoctonia solani

Soil-inhabiting fauna or fungi might be implicated in the spread of virus underground. Among common soil fungi which attack potato plants are species of *Verticillium* and *Rhizoctonia*. Blair (1943) showed that *R. solani* could grow 21–24 cm. through natural unsterilized soil, so this fungus was chosen as a connecting link between individual tubers. The method used was to inoculate *X*-infected half-tubers in the laboratory with the fungus, and to pair these with healthy half-tubers out of contact, but within a suitable distance for the fungus to form a bridge between them. The trays containing the tubers were incubated, and 6 days after connexion by the fungal hyphae had been established the tuber pieces were potted, and the shoots afterwards tested for *X*. One such test made with infected Majestic tubers gave negative results, as did another made on fourteen Kerr's Pink tubers. Negative results were also obtained in an experiment described previously in this paper in which *X*-infected tubers, deliberately infected with the fungus, were planted in sterilized soil in the same pots as healthy tubers. On the basis of these results it is thought to be unlikely that fungi are concerned in the spread of virus *X*, but more extensive testing is necessary before the possibility can be excluded.

Discussion

Because of the widespread occurrence of potato virus *X* in most varieties of potato, it is often assumed that the virus spreads freely. Under the conditions of the experiments described here, however, spread is much less rapid than with the aphis-transmitted viruses that cause leaf roll and rugose mosaic. There seem to be considerable differences between the rate at which different strains of *X* spread, those that reach the highest concentration in infected plants spreading most rapidly, but in only one experiment did more than 10% of the plants exposed to sources of infection in the field become infected in one growing season. In the course of the experiments no infections have been obtained that could not be explained by healthy plants coming into contact with sources of infection, either infected plants, residues or sap from infected plants. Hansen (1941) expressed doubt that leaf contact and 'wind mutilation' could explain the occurrence of virus *X* and considered that he had encountered examples of 'auto-infection'. Nothing has been observed in these experiments, either in the field or under glass, to suggest that the virus can arise spontaneously in previously healthy potatoes. The results, however, do show that spread by leaf contact is only part of the story.

In tomato plants spread occurs much more freely than in potatoes, and with this host it was easy to demonstrate that spread can occur when only underground parts of healthy and infected plants were in contact. With potatoes this was less easy to demonstrate, though one unequivocal example of spread below ground was obtained under glass. It seems likely that in the field this method is also responsible for as much spread as leaf contact, though it remains unproven. The fact that many plants whose foliage gave no reaction for virus *X* at the end of the growing season had some infected tubers provides the strongest evidence for this. Clinch (1944) also quotes examples of potato plants with apparently virus-free foliage yielding a mixed progeny of healthy and infected tubers. She interpreted this as evidence that infection of the foliage had occurred late in the season, when the translocation of food substances was mainly towards the tubers, and that the virus moved with the stream of foodstuffs from the originally infected leaf to the tuber without invading the rest of the haulm. There is, however, no experimental evidence that such unilateral movement of virus does occur, and when old plants are artificially infected the youngest leaves as well as the tubers become infected. It seems more likely that these mixed progenies from plants with apparently virus-free haulms arise because of infection occurring only on parts of the root or stolon system. The mechanism of underground spread is not understood. The simplest explanation is that it occurs as a result of mechanical contact between healthy and infected tissues, but the possibility of soil flora or fauna being involved cannot be excluded.

A point that should be stressed in work with potato virus *X* is that unless potato plants are known not to have been in contact with any disease source, individual tests on the tubers are necessary to ensure that they are virus free; tests on the foliage of the parent plants alone are not reliable for this purpose.

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(*Received* 31 *July* 1947)

RESOLUTION OF STRAWBERRY VIRUS COMPLEXES

II. VIRUS 2 (MILD YELLOW-EDGE VIRUS)

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(With Plate 6)

Aphids (*Capitophorus fragariae* Theob.) allowed to feed for several days on a strawberry plant infected with yellow-edge transmitted two virus fractions. The isolation and properties of one (virus 1) have been described previously. The other (virus 2) was separated by transferring the aphids to fresh indicators after 24 hr.

Virus 2 was retransmitted after infection feeding periods of 24 hr. or more and persisted in the vector for several days. There is some evidence that it is itself a complex of viruses which can be separated further. On *Fragaria vesca* virus 2 produced chlorotic spotting, slight marginal chlorosis of the leaves and slight cupping of the leaflets. On Royal Sovereign strawberry it produced slight chlorosis of the young leaves.

On Royal Sovereign viruses 1 and 2 together produced symptoms of yellow-edge which is thus shown to be caused by a virus complex which can be resolved by means of the aphis vector.

Introduction

It has been shown (Prentice & Harris, 1946) that strawberry aphids (*Capitophorus fragariae* Theob.) feeding for periods of 24 hr. on strawberry plants infected with mild crinkle, severe crinkle or yellow-edge, transmit what appears to be one virus only, when they are transferred to virus-free plants. This virus, which was believed by the authors cited to be the mild crinkle virus, will be referred to in the present paper as virus 1. In the same communication it was suggested that the failure to transmit other viruses, presumed to be present in plants infected with yellow-edge and severe crinkle, was due to the shortness of the feeding times employed.

Using the same species (*Capitophorus fragariae*) but with longer infection feeding periods, it has now been found possible to separate another virus component from plants infected with yellow-edge. The methods employed, and experiments on the properties and aphis relationships of the virus isolated are described below. A preliminary account of some of the results obtained has already been published elsewhere (Prentice, 1946).

In the present experiments a simplified form of the technique previously used (Prentice & Harris, 1946) was employed. Aphids were not subjected to a period of fasting and, instead of detached leaves, complete plants were used as sources of infection. This was found necessary owing to the longer feeding times used. Stocks of *C. fragariae* Theob. for use in the experiments were raised on clonal plants (i.e. plants raised vegetatively from one original parent plant) of the wild strawberry (*Fragaria vesca* L.) or of the cultivated strawberry (var. Royal Sovereign, Malling 40 clone) showing no symptoms and believed to be virus free. Aphids were transferred from these stocks to infected strawberry plants by means of a moistened camel-hair

brush, and, after the appropriate period, retransferred to virus-free 'indicator' plants. The infected plant on which aphids fed in a particular experiment will be referred to as the *infector* and the period for which aphids were allowed to feed on the infector as the *infection feeding period.*

As the life of an individual apterous *Capitophorus fragariae* is normally only a few weeks, it was found that after long infection feeding periods the mortality among aphids during subsequent transfers was high. Infection feeding periods of individual aphids were therefore limited to about 14 days. In some experiments, aphids were allowed to multiply on the infector for long periods and some of the progeny were subsequently transferred. In such cases the infection feeding period of the individual aphids was not accurately determined, being the same as the age of the aphid transferred. The period for which such aphis stocks were allowed to feed and multiply on the infector will be referred to as the *infection feeding period of the stock.*

In most cases aphids, after feeding for a period on one indicator, were retransferred to a fresh indicator and subsequently again retransferred. The period for which aphids were allowed to feed on the first set of indicators in an experiment will be called the *1st transfer feed*, the period on the second set (to which aphids were retransferred) the *2nd transfer feed*, and so on.

The number of plants infected in each experiment will be shown as a ratio, the numerator being the number of indicator plants developing symptoms of infection and the denominator the total number of indicators receiving a particular treatment. All references to 'aphids' are to apterous *C. fragariae.*

Indicators were dipped in a nicotine insecticide mixture after aphids had been transferred from them. Where aphids were not retransferred, the indicators were similarly dipped after the completion of the appropriate feeding period. All plants were grown in pots in the glasshouse and sprayed weekly with an insecticide except when aphids were being allowed to feed on them. Controls receiving aphids direct from the aphis stock and controls receiving no aphids were kept for each experiment. None of these became infected.

EXPERIMENTAL

(i) *Isolation of virus 2 and symptoms on* Fragaria vesca

Exp. 1. *May* 1943 (see Table 1)

TABLE 1

*Infector:** Royal Sovereign infected with yellow-edge.
Infection feeding period of stock: 8 weeks.
Indicator: F. vesca. Two aphids per indicator.

Transfer	Period	Plants infected
1	10 min.	0/4
2	24 hr.	4/4
3	24 hr.	2/4
4	8 days	2/4

The infector used in this experiment was of the same clone as the yellow-edge infected plants used in experiments already described (Prentice & Harris, 1946).

* For explanation of terms employed see above (Introduction).

Symptoms similar to those already described for virus 1 developed on the indicators of the 2nd transfer feed after 14–20 days. The symptoms on the indicators of the 3rd and 4th transfer feeds developed after about 8 weeks and were of a different type. Small chlorotic flecks appeared on the young leaves, chiefly on the smaller leaf veins. The leaflets then became slightly concave and slightly chlorotic especially towards the margins and the plants gradually lost vigour (Pl. 6, fig. 1).

The virus (or viruses) producing these symptoms is apparently different from virus 1. It has been referred to previously as the mild yellow-edge virus (Prentice, 1946) and will be referred to here as virus 2.

Exp. 2. *July* 1943 (see Table 2)

TABLE 2

Infector: Royal Sovereign infected with yellow-edge (same clone as Exp. 1).
Infection feeding period: 10 days.
Indicator: F. vesca. Four aphids per indicator.

Transfer	Period	Plants infected
1	48 hr.	3/4
2	48 hr.	0/4
3	48 hr.	1/4
4	48 hr.	1/4

The symptoms on the plants of the 1st transfer feed appeared in 17–21 days and were similar to those produced by virus 1. Symptoms developed on plants of the 3rd and 4th transfer feeds in about 7 weeks and resembled those in the 3rd and 4th transfer plants in Exp. 1 (i.e. symptoms produced by virus 2).

(ii) *Transmission*

Exp. 3. *August* 1943 (see Table 3)

TABLE 3

Infector: F. vesca (from Exp. 1, 4th transfer) infected with virus 2.
Infection feeding periods: Various.
Indicator: F. vesca. Four aphids per indicator. Single transfer 3 days.

Infection feeding period	Plants infected
2 hr.	0/4
24 hr.	1/4
5 days	4/4
21 days	3/3

Exp. 4. 1944–5 (see Table 4)

TABLE 4

Infector: F. vesca infected with virus 2.
Infection feeding periods: Various.
Indicator: F. vesca. Five aphids per indicator. Single transfer 24 hr.

Infection feeding period	Plants infected
6 hr.	0/5
24 hr.	1/15
2 days	4/6
3 days	1/4

The symptoms produced on the indicators in these experiments were similar to those caused by virus 2 and developed in 4–7 weeks.

Exp. 5. 1946

The results of further trials to determine the infection feeding time necessary to obtain transmission of virus 2 to *F. vesca* are shown in Table 5. In this experiment, infected Royal Sovereign or infected *F. vesca* plants were used as sources of virus 2 and one or five aphids were transferred to each indicator for 24 hr. The Royal Sovereign infectors were the progeny of plants infected with virus 2 by grafting as described below (§ (iii)). In Exp. 5, variation was noted in the symptoms developed by infected indicators, but discussion of this will be postponed to a later section (§ (viii)). It will be seen that the proportion of infections was higher in these experiments when *F. vesca* was used as infector, but no explanation of this is offered.

TABLE 5. *Transmission of virus* 2 *after different infection feeding times*

Infector: Royal Sovereign or *F. vesca* infected with virus 2.
Infection feeding period: Various.
Indicator: F. vesca. One or five aphids per indicator. Single transfer 24 hr.

		Infection feeding period		
	Infector	1 day	2 days	3 days
One aphid per indicator:	Royal Sovereign	0/5	0/5	0/5
	F. vesca	0/5	3/5	2/5
Five aphids per indicator:	Royal Sovereign	0/5	0/5	1/5
	F. vesca	0/10	3/10	9/15

When the results of Exps. 3–5 are considered together, it appears that virus 2 is occasionally transmitted after an infection feeding period of 24 hr., but that more infections are obtained after feeding periods of several days.

(iii) *Symptoms on Royal Sovereign*

In June 1944, two of the infected *F. vesca* plants from Exp. 3 were each grafted by the stolon inarching method (Harris & King, 1942) to a Royal Sovereign strawberry plant. No symptoms were detected on the Royal Sovereign plants during 1944 and they were propagated by taking runners from them in 1945. In autumn 1945 the runners and parent plants all developed symptoms of a very mild 'yellow-edge' type. The younger leaves were slightly chlorotic, especially towards the margins, and the petioles were slightly shorter than normal. There was, however, no obvious dwarfing or distortion of the laminae (Pl. 6, fig. 2, top right). These plants appeared almost normal during the summer of 1946, but symptoms were again noticeable in the autumn and were then more conspicuous than in 1945. Runners from infected plants were planted out-of-doors and developed slight but definite symptoms of yellow-edge.

Two plants of *F. vesca* infected with virus 2 and two runners from the Royal

Sovereign plants infected as above were grafted to healthy *F. vesca* plants. Typical symptoms of infection with virus 2 developed on the indicators in 6–8 weeks.

In later trials, Royal Sovereign plants grafted to Royal Sovereign or *F. vesca* plants infected with virus 2 developed mild symptoms in about 8 weeks.

(iv) *Persistence*

The occurrence of virus 2 in the 4th transfer indicators in Exp. 1 (above) shows that this virus persists in the vector for at least 48 hr. Other experiments were made in 1945 and 1946 to obtain more information on the persistence of the virus.

Exp. 6 (see Table 6)

TABLE 6

Infector: F. vesca infected with virus 2.
Infection feeding period of stock: 3 weeks.
Indicator: F. vesca. Five aphids per indicator.

Transfer	Period	Plants infected
1	24 hr.	5/5
2	48 hr.	5/5
3	48 hr.	5/5

Exp. 7 (data from several trials)

The results of experiments on persistence made in 1946 are shown in Table 7. In this experiment, variation was again noticed in the symptoms exhibited by the indicators (see § (viii)).

TABLE 7. *Persistence of virus 2*

Infector: Royal Sovereign infected with virus 2 by grafting.
Infection feeding period of stock: 14 days. Aphids 4–5 days old.
Indicator: F. vesca. One or five aphids per indicator.

		One aphid per indicator Trial			Five aphids per indicator Trial		
Transfer	Period	A	B	C	A	B	C
1	48 hr.	1/5	1/5	2/5	3/5	5/5	3/5
2	48 hr.	1/2*	3/5	2/5	2/5	4/5	4/5
3	48 hr.	0/3	0/5	0/4*	2/5	0/2	2/5
4	48 hr.	—	—	2/5	—	—	4/5†
5	48 hr.	—	—	1/5	—	—	1/1
6	48 hr.	—	—	—	—	—	1/1
7	48 hr.	—	—	—	—	—	1/1

* One indicator died from accidental causes.
† All aphids remaining at this stage (about 20) transferred to one indicator.

It will be seen from Table 7 that aphids were still infective after 6–8 days, and that some were still infective after 12 days. It is probable that, in fact, some aphids remain infective for the remainder of their lives. It appears also that an aphid may infect the second or later plant to which it is transferred although it has failed to infect the first.

(v) *Presence in other infected plants*

Exp. 8

Attempts were made to demonstrate the presence of virus 2 in other infected plants. The results are summarized in Table 8. The symptoms produced at first on

TABLE 8. *Attempted transmission of virus 2 from different infectors*

Infector: Various plants infected with yellow-edge.
Infection feeding period: 10 days.
Indicator: F. vesca. Five aphids per indicator. 1st transfer feed 24 hr. 2nd transfer feed 3 days.

Infector	Plants infected: 1st transfer (virus 1)	Plants infected: 2nd transfer (virus 2)
1. Royal Sovereign of clone used in earlier experiments (infected with yellow-edge)	10/15	4/15
2. Royal Sovereign of another clone (also infected with yellow-edge)	4/5	3/5
3. Seedling (yellow-edge carrier)	5/5	0/5
4. Early Cambridge (yellow-edge carrier)	3/5	0/5

the *F. vesca* indicators of the 1st transfer feed were typical of those produced by virus 1. Later the symptoms on two of the ten indicators infected from Royal Sovereign as used in earlier experiments became modified so as to resemble symptoms produced on *F. vesca* by yellow-edge in grafting experiments. The leaves on these plants developed marginal chlorosis and were reduced in size. Symptoms on indicators of the 2nd transfer feed resembled those produced by virus 2.

In a trial similar to those recorded in Table 8, but using a 'degenerate' plant of the variety Huxley's Giant (Prentice, King & Harris, 1946) as infector, all five *F. vesca* indicator plants of the 1st transfer feed at first developed symptoms of infection with virus 1. Three of these, however, later developed modified symptoms as described above. Three of the indicators of the 2nd series were infected, but the symptoms exhibited by them were milder than those produced by virus 2, the chlorosis and leaf cupping was less pronounced and chlorotic spotting was absent.

Similar results were obtained using aphids from a stock which had fed for 3 months on a Huxley plant 'carrying' yellow-edge. All ten indicators of the 1st transfer developed symptoms of infection with virus 1, but these later became modified on four plants. Five of the ten plants of the 2nd transfer developed mild symptoms resembling those obtained in the experiment just described.

These mild symptoms were retransmitted by *Capitophorus fragariae* after an infection feeding period of 3 days.

(vi) *Resynthesis of yellow-edge*

As has been previously described, Royal Sovereign plants infected with virus 1 develop faint chlorotic spotting only, and this is often so faint as to be scarcely distinguishable (Prentice & Harris, 1946). As stated above, Royal Sovereign plants infected with virus 2 are very slightly reduced in vigour and their younger leaves are slightly chlorotic, particularly towards the margin.

In May 1945, two Royal Sovereign plants infected with virus 1 were each grafted to a Royal Sovereign plant infected with virus 2. In October, all four plants showed symptoms of severe yellow-edge. In July 1946, four similar pairs were grafted and all developed symptoms of severe yellow-edge. Symptoms appeared first (in about 5 weeks) on the plants originally infected with virus 2 and later (in about 2 months) on the plants originally infected with virus 1. One plant of *F. vesca* infected with virus 2 was grafted to a Royal Sovereign plant infected with virus 1. The Royal Sovereign developed severe yellow-edge and the *F. vesca* plant became severely dwarfed and finally died. A plant of *F. vesca* grafted to a Royal Sovereign infected with viruses 1 and 2 died similarly.

The combination of viruses 1 and 2 by means of aphids also produced yellow-edge. Transfer of five aphids fed for 4 days on a plant infected with virus 2 to each of five Royal Sovereign plants infected with virus 1 caused two of the latter to develop definite symptoms of yellow-edge.

The production of yellow-edge symptoms by combining viruses 1 and 2 was obtained using either the mild or the severe symptom type of virus 1. These types have been previously described (Prentice & Harris, 1946).

(vii) *Effect on Huxley's Giant strawberry*

No virus-free plants of the variety Huxley's Giant (a yellow-edge tolerant variety) were available, so that it was not possible to investigate the effect of virus 2 alone. Huxley plants (Malling 44 clone) known to be infected with 'mild crinkle' (i.e. containing virus 1 and possibly another virus or viruses in addition, but not containing virus 2) were grafted to Royal Sovereign or *F. vesca* plants infected with virus 2. In a number of cases the grafts failed, but in two cases in 1945 grafts of Royal Sovereign to Huxley were successful. Both of the Royal Sovereign plants developed symptoms of severe yellow-edge in about 3 months, and both of the Huxleys developed symptoms in the following season similar to those shown by 'degenerate' plants (Prentice *et al.* 1946). Two grafts of *F. vesca* (infected with virus 2) to Huxley were successful in 1946. Both of the *F. vesca* plants became severely dwarfed and one subsequently died. Both of the Huxleys developed incipient symptoms of degeneration in the autumn (5 months after grafting) and definite symptoms in the following year.

In an attempt to transmit virus 2 to Huxley by means of aphids, five aphids from a stock fed for 3 weeks on a Royal Sovereign plant infected with virus 2 were

transferred to each of five Huxley plants for 3 days. Two of the Huxley plants developed symptoms of 'degeneration' in the following autumn.

(viii) *Variation in symptoms*

In the 1946 experiments, as has been mentioned above (§§ (ii) and (iv)), variation was noted for the first time in the symptoms developed by *F. vesca* plants to which aphids had been transferred after feeding on plants infected with virus 2. Some of these *F. vesca* indicators developed symptoms which were much milder than those which have been described as being typical of virus 2 infection. Cupping and marginal chlorosis were almost entirely absent and symptoms were reduced to a slight but definite chlorotic mottling, some intervenal necrosis on the older leaves and slight loss of vigour. This differentiation into two symptom types was noted in a number of experiments during 1946, and it may be that the fraction here described as virus 2 is itself a complex capable of further resolution.

As has also been described above (§ (v)) symptoms somewhat resembling those produced by virus 2, but believed to be distinct, were obtained in experiments in which Huxley plants were used as infectors. The younger leaves were chlorotic, especially towards the margins, and the plants were reduced in vigour but chlorotic spotting was absent.

(ix) *Attempted direct transmission of yellow-edge*

In a number of different experiments, aphids which had fed for 5, 7 or 10 days or aphids from stocks which had fed for 2, 3, 12 or 24 weeks on plants infected with yellow-edge were transferred to virus-free Royal Sovereign plants. Five, ten or twenty aphids were transferred to each indicator in different experiments and in some cases the aphids were retransferred to fresh indicators after 24 or 48 hr. A number of the Royal Sovereign plants developed symptoms of vein-clearing or chlorotic spotting which in some cases was quite conspicuous and in other cases faint and indefinite. Some of the plants showed very slight symptoms suggestive of yellow-edge, but these were too indefinite for positive diagnosis. A number of these plants were retained until the following year and propagated and while, in a few cases, mild symptoms developed which resembled those caused by virus 2, in no case were definite symptoms of yellow-edge noted.

DISCUSSION AND CONCLUSIONS

It has been shown previously that one virus component (here called virus 1) can be isolated from a plant infected with yellow-edge and it has now been shown that a second component (virus 2) can also be isolated. Virus 2 is dealt with, for the present, as a single virus, although there is some evidence that it may be capable of further resolution.

Virus 1 and virus 2 differ in many respects. Virus 1 is transmitted occasionally after an infection feeding period of 1 hr. and is readily transmitted with infection

feeding periods of 24 hr. Virus 2 is transmitted occasionally after an infection feeding period of 24 hr., but a greater number of infections is obtained with feeding periods of 3–4 days. Virus 1 persists for only a few hours in the vector, whereas virus 2 persists for several days.

Symptoms appear on *Fragaria vesca* in 2–3 weeks with virus 1 and in 4–8 weeks with virus 2. The symptoms produced by virus 2 are somewhat similar to those produced by the mild symptom type of virus 1, but virus 2 can generally be distinguished by the leaf cupping and marginal chlorosis which it produces. On Royal Sovereign virus 1 produces a faint chlorotic spotting which is sometimes difficult to detect; virus 2 produces a slight leaf chlorosis, tending to be marginal, but this is difficult or impossible to detect on recently infected plants.

Viruses 1 and 2 are therefore considered to be distinct since they differ in their vector relationships, in the incubation period of the diseases which they cause and in the symptoms which they produce. Although virus 1 is less persistent than virus 2, both viruses appear to belong to the 'persistent' type (Watson, 1946).

Virus 2 can be transmitted from *F. vesca* to Royal Sovereign or vice versa by grafting, with the consistent production of appropriate symptoms, but in a number of aphis experiments carried out in 1946 two types of symptom were produced in transfers to *F. vesca* from plants infected with virus 2. It may be, therefore, that virus 2 is itself a complex capable of further resolution and, on this hypothesis, the milder symptom type occurring in these experiments (slight chlorotic spotting and necrosis without obvious cupping or general chlorosis) may be considered to be produced by one component of the virus 2 complex. A reasonable corollary to this, but one unsupported by experimental evidence, would be that the persistent virus obtained from infected Huxley plants (§ (v) above) is the complementary fraction of virus 2. This virus produces general chlorosis and slight cupping but no chlorotic spotting on *F. vesca*. An alternative explanation of the two symptom types obtained from virus 2 infectors is that the mild symptoms are due merely to the isolation of a milder strain of virus 2 and for the present, therefore, virus 2 is considered as a single virus.

The combination of virus 1 and virus 2 by grafting produces very severe symptoms on *F. vesca*. Chlorotic spotting and slight marginal chlorosis are combined, the young leaves are much reduced in size, the petioles are shortened and the whole plant is much reduced in vigour and may be killed. The combination of the two viruses in Royal Sovereign produces symptoms of a severe form of yellow-edge (Pl. 6, fig. 2). The leaves produced after infection are reduced both in size of laminae and length of petiole and the margins of the younger leaves are chlorotic, especially in the autumn. The greater severity of yellow-edge symptoms in autumn has already been noted (King & Harris, 1942).

It will thus be seen that a severe form of yellow-edge can be produced in Royal Sovereign by the combination of two viruses each of which, when acting singly, produces very mild symptoms on this variety. It is therefore established that this

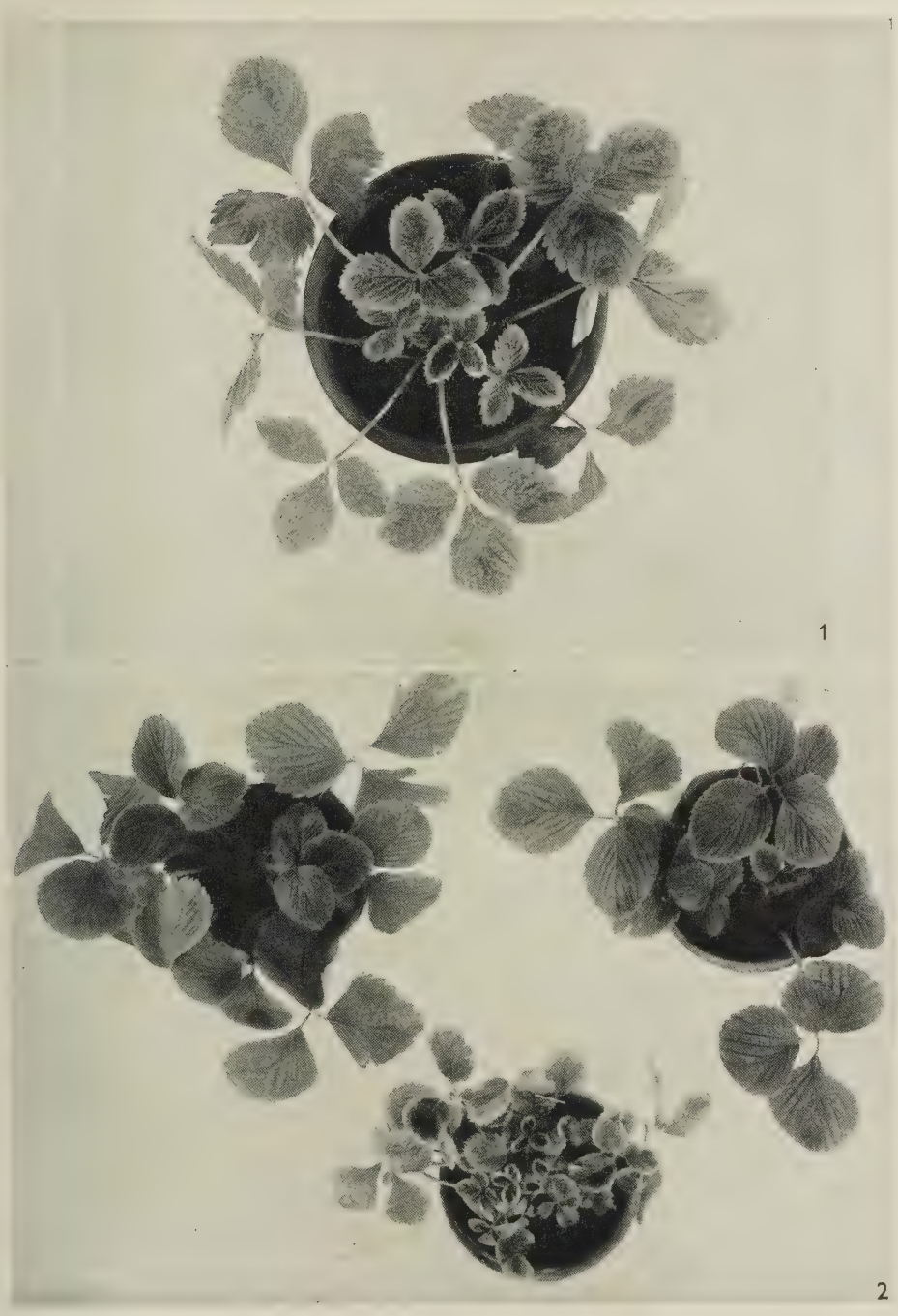
form of yellow-edge is a complex disease and that the occurrence of virus 1 (which is etiologically concerned in the production of 'mild crinkle') in such yellow-edge infected plants is not merely fortuitous. The constant occurrence of crinkle in plants infected with yellow-edge had already been noted in grafting experiments (Harris & King, 1942).

The repeated failure to obtain direct transmission of yellow-edge to Royal Sovereign plants is difficult to explain. It would, however, appear from the comparatively small number of *F. vesca* plants which were infected with virus 2 when yellow-edge infected Royal Sovereign plants were used as infectors, that these Royal Sovereign plants were not a good source of virus 2. All of them had been infected for a considerable period, being runners of infected clones which had been maintained for several years. Even so, it seems likely that virus 2 was transmitted to some of the Royal Sovereign indicator plants and, as virus 1 is readily transmitted, many of the indicators must have been infected with that virus. It is remarkable that the combination of virus 1 and virus 2 did not apparently occur in the experiments mentioned. Previous workers in this field have successfully transmitted yellow-edge by direct transfer of aphids (Chamberlain, 1934; Massee, 1935, 1936, 1937) and xanthosis, which is probably identical with yellow-edge, has been similarly transmitted (Plakidas, 1927).

Virus 1 and virus 2 may have been combined in some of the *F. vesca* indicators in experiments described above (§ (v)), as in some cases 1st transfer indicators developed symptoms resembling those produced on this species when grafted to plants infected with yellow-edge. The stolons produced by these indicators, however, were extremely short and thin and efforts to graft them to Royal Sovereign plants, and thus to demonstrate that they were infected with yellow-edge, failed.

The addition of virus 2 to plants of Huxley's Giant, which is a yellow-edge-tolerant variety (Rogers, King & Massee, 1939) produces symptoms of 'degeneration', that is, reduction in vigour and slight chlorosis of the leaf margins, especially in the autumn. The Huxley plants used were already infected with virus 1 and possibly with other viruses in addition and it is, therefore, not clear whether the 'degeneration' was caused by virus 2 or by the virus complex formed. The symptoms of yellow-edge which developed on the Royal Sovereign plants used to infect these Huxley plants are presumably due to the former becoming reciprocally infected with virus 1 from the latter and the consequent production of yellow-edge in the Royal Sovereigns by the combined action of virus 1 and virus 2.

Thus the relationship of 'yellow-edge' in Royal Sovereign to 'degeneration' in Huxley remains undecided. It is probable, however, that the degeneration of Huxley induced by grafting with yellow-edge infected Royal Sovereign plants (Prentice *et al.* 1946) was due to the introduction of virus 2. If the hypothesis that virus 2 is itself a complex is substantiated by further experiment this may help to elucidate the relation of yellow-edge to degeneration.



PRENTICE—*Resolution of strawberry virus complexes*

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EXPLANATION OF PLATE 6

Fig. 1. *Fragaria vesca* plant infected with virus 2.

Fig. 2. Top left: Royal Sovereign infected with virus 1 (symptoms not visible in photograph). Top right: Royal Sovereign infected with virus 2 (slight chlorosis on young leaves). Bottom: Royal Sovereign infected with virus 1 and virus 2 (severe yellow-edge).

(*Received* 16 *August* 1947)

SOME NOTES ON *HOLCUS MOLLIS* L.

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The natural woodland habitat of *Holcus mollis* is discussed and its ability to survive after removal of the trees. Its presence under other environmental conditions is recorded. Examples of its occurrence as a weed are quoted from Scotland, including an instance of competition with an oat crop. The effect of grazing and cultivation on *H. mollis* is described.

A recent interesting article by Mann & Barnes (1947) dealing with *H. mollis* as a weed of barley raises the question of how this grass becomes a weed. This can only be answered by investigating the natural habitat of *H. mollis*, and its reaction to change of environment.

H. mollis (creeping soft grass) is naturally a shade-loving grass which is frequent and sometimes very common in both pedunculate and sessile oak woods (Fenton, 1941). It also occurs under beech—provided the shade is not too great—but is less common in ash woods. In pine woods (e.g. pure stands of Scots pine) it is comparatively rare and is found only near paths or tracks, but where birch is also present *H. mollis* is by no means uncommon. Further references to these matters are given by Tansley (1939).

In rather open coniferous woods in Scotland, *H. mollis* occurs in patches. This seems to contradict the previous statement, but the grass is found where either non-coniferous woods or mixed woods once occupied the ground, and it has persisted in spite of the change in the nature of the wood (Fenton, 1943). *H. mollis* is not unknown as a constituent of grassy heaths, where probably there had been at one time a scrubby growth of trees or shrubs forming a very open woodland. On the rough (hill) grazings in south-east Scotland it is mostly found in or near bushes of *Ulex europaeus*. When these bushes are periodically burned, *Holcus mollis* temporarily covers the bare ground with a dense growth, until slowly crowded out by the recovering *Ulex*. After this the grass still continues on the fringe of the *Ulex* community and may even spread slowly outwards.

Holcus mollis and wavy hair grass (*Deschampsia flexuosa*) are frequently associated with bracken (*Pteridium aquilinum*). Both grasses endure much shading but *Holcus* is capable of reaching a height of over 2½ ft., and thus may survive conditions which repress wavy hair grass. Once *H. mollis* establishes itself in this environment, it is not easy to eradicate. It is apparent that its ability to survive by means of its underground stem, to endure shade and also to grow to some height if mixed with other tall-growing vegetation, enables it to compete very successfully with other plants.

As much of our arable land was, originally, derived from forest it is not surprising that *H. mollis* occasionally occurs as a weed of cultivated ground. Fortunately it is not a very common weed in most parts of Britain. It is, like *H. lanatus* (Yorkshire fog grass), tolerant of a wide range of soils, but where *H. mollis* occurs in woods it does not seem to recover very quickly after grazing. In this respect it again resembles *H. lanatus* which is suppressed on grazed pastures of reasonable fertility by more rapidly growing grasses and clovers (Fenton, 1931). On poorer soils it does not always compete successfully with *Agrostis* spp. (bent) or *Deschampsia flexuosa*. Generally it is a relict (Clements, 1934) from what had formerly been a woodland type of vegetation.

A very interesting example of *Holcus mollis* becoming a weed and causing damage to an oat crop in Scotland will illustrate some of the points discussed. The instance occurred on a light loam soil at an elevation of 800 ft. with a south-eastern aspect. The land had once been woodland, mostly, if not entirely, non-coniferous, and subsequently a permanent pasture of the *Agrostis* (bent) type. In the upper part occasional blades of *Holcus mollis* were found but it was not plentiful.

The field was ploughed and laid down to grass of a better type during the 1939–45 war. This was broken up and an oat crop sown. The upper half of the field under the oat crop produced an amazingly dense growth of *H. mollis* which seriously reduced the crop. As a result, this half has since been left, except for a strip of potatoes on the north side, and is now a dense growth of *H. mollis* except for a few patches where *Agrostis* is dominant. The lower half of the field was sown down to turnips and swedes. At the edge of these crops, *Holcus* has depressed the yield in a few small patches, but elsewhere the crop is free from grass. Again, next the potato patch *H. mollis* is dense, but the potato drills themselves are free from grass or weeds. Another small patch in the upper part, which has been ploughed this spring (1947) and since neglected, is completely dominated by *Holcus*. But the growth in this part is much less dense than in the unploughed area.

Immediately above this field and separated from it by a dry stone dyke (wall) is an area felled about 1914–18. The wood, as far as can be assessed now, was mixed, with patches of conifers. Since it was felled, the whole area has become derelict. For the most part the ground is dominated by a dense growth of *H. mollis*, except for a few parts where *Deschampsia flexuosa* is the dominant grass with some *Agrostis*. Rabbits have intruded into parts of this area, and round their burrows for a distance of several feet there is no *Holcus*. It is replaced chiefly by *D. flexuosa*, *Agrostis* and *Galium saxatile*.

From these notes it is apparent that *Holcus mollis* may become a noxious weed, but there are ways and means of holding it in check.

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(*Received* 13 *October* 1947)

OBITUARY NOTICE

ROBERT STEWART MACDOUGALL

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An original member of the Association, 1904–47, *a member of Council*, 1908–13, *Vice-President*, 1914–19

Like John Curtis, Eleanor Ormerod and Fred Theobald, Stewart MacDougall stands out as a pioneer of economic entomology. Renowned as a teacher, he became no less famous as a consultant. In the development of entomology in Scotland his influence was decisive.

Robert Stewart MacDougall, born in 1862 and educated at George Heriot's School and at the Universities of Edinburgh and Munich, was a university teacher in Edinburgh for almost forty years. His lectures to veterinary and other students started soon after his graduation in 1890: his appointment as Professor of Biology at the Royal (Dick) Veterinary College was made in 1903. In 1906 he became Steven Lecturer in Agricultural Entomology in the University of Edinburgh, and two years later this Lectureship was extended to include Forest Entomology. In 1908 also he was appointed as temporary Lecturer in Botany. From 1920 until his retirement in 1929 he held the Readership in Agricultural and Forest Entomology.

Dr MacDougall's services extended far beyond his university appointments. From 1904 to 1913 he acted as Technical Adviser in Zoology to the Board of Agriculture and Fisheries, and for thirty-six years he was Consulting Zoologist to the Highland and Agricultural Society of Scotland. The Scottish Board of Agriculture made him their Adviser in Biology, and he served as a member of the Scientific and Technical Commission of the International Institute of Agriculture in Rome. The Botanical Society of Edinburgh elected him as President for 1913–15, and he held office as Vice-President of the Royal Entomological Society and on the Committee of the Imperial Bureau of Entomology.

His activities had wide and far-reaching effects on the growth of applied entomology. The long series of articles contributed to the *Journal of the Board of Agriculture* in the early years of this century sowed the seed that developed and fructified in 1911 as the advisory entomological service, linked with the universities. Through the achievements of his old students the recognition of his greatness as a teacher has become world-wide.

He had the gift of securing the utmost confidence of those with whom he worked and of encouraging them to high attainment. Prof. Russell Greig has selected a fitting estimation of Stewart MacDougall from the works of Carlyle: 'A man of lively intellectual faculties, of ardent friendly character, and of wide speculation and acquirement; very fearless, very kindly, without ill-humour and without guile.'

(This notice is written from information kindly supplied by Dr A. E. Cameron, by Dr C. B. Williams, and by the Deputy Secretary of Edinburgh University. Grateful acknowledgement is made to Prof. Russell Greig for permission to include the final sentence.)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Annual General Meeting of the Association held on Friday, 20 February 1948, in the Imperial College of Science and Technology, London; the President, Mr W. C. Moore, in the Chair.

After the formal business, the following papers were read and discussed:

Stored-products pests: a survey of the principal entomological problems in the United Kingdom. By Dr J. A Freeman.

Stored-products pests: a survey of methods of control in the United Kingdom since 1939. By Dr E. E. Turtle.

STORED-PRODUCTS PESTS: A SURVEY OF THE PRINCIPAL ENTOMOLOGICAL PROBLEMS IN THE UNITED KINGDOM

By J. A. Freeman, *Ministry of Agriculture Infestation Division, 58 High Holborn, London, W.C. 1*

(With 2 Text-figures)

At the outbreak of war in 1939, basic scientific knowledge had been obtained in the field of industrial entomology in this country as the result of the work at Imperial College under Prof. J. W. Munro. Although detailed studies of infestations in the tobacco (Bovingdon, 1933; Freeman & Yapp, 1940) cocoa (Munro & Thomson, 1929; Herford & Richards, 1930) and dried fruit trades (Myers, 1928; Herford & Richards, 1930) had been carried out, a survey of conditions in the grain trade had only just been completed and the results had not been collated. In this matter we had not advanced far beyond the reports of the Royal Society Grain (War) Pests Committee of 1918–21. Opportunity had been taken during the grain pest survey (Munro, 1940) to inspect the reserve stocks of wheat placed in store by the Government in 1938, and advice had been given to those commercially responsible for its storage. But the country was ill-equipped, both in scientific and operational man-power, for dealing with any major outbreaks of infestation in stored products.

Although no serious outbreaks of infestation had occurred during 1940, the Ministry of Food appointed two entomologists that autumn to carry out an entomological survey of the security wheat stocks, one of these entomologists being seconded from the Pest Infestation Laboratory set up in May 1940 by the Department of Scientific and Industrial Research. During 1941 these entomologists extended their survey to other commodities, but their function was confined to advising the local and headquarters officers of the Food Ministry, as there was no executive organization for control action. Towards the end of the summer of 1941 there occurred two serious insect outbreaks, which were of such severity that the Ministry found it necessary to establish an executive and inspectorial organization based on legal powers (Statutory Instruments, 1941, 1943, 1945). This organization was established in March 1942 as the Infestation Branch of the Ministry of Food.

Two species of insects were responsible. The major outbreak, which caused particular alarm to those responsible for the custody of the grain stocks, was the appearance of large numbers of migrating caterpillars of the cacao moth (*Ephestia elutella* Hb.), with associated webbing on the surface of the stocks of bagged and bulk security wheat reserves at the end of August 1941. The outbreaks took place in the main ports in England, but not in Scotland or Northern Ireland. During the previous year and early in 1941, small numbers of caterpillars had been observed spun up on the surface of grain and in the fabric of the warehouses, but none of the original survey entomologists had anticipated such a rapid increase in numbers. Even if large numbers of moths had been observed during the summer of 1941, it would have been difficult to take any action as the apparatus and organization necessary did not exist. The other outbreak was of *Oryzaephilus surinamensis* L. on dried fruit. The routine fumigation on arrival which had been the practice on Australian dried fruit for many years was not applied to fruit from other countries. Towards the end of 1941 there were severe outbreaks of *Oryzaephilus surinamensis* (saw-toothed grain beetle) in dried fruit stored in Liverpool and London. Immediate control measures had to be applied, and as a long-term measure it was decided to fumigate all dried fruit on arrival as a routine measure, whether or not it was known to be infested. An interesting feature of both outbreaks is that they did not occur until about two years after the foodstuffs had been put into store; in the *Ephestia elutella* attack it is fairly certain that the infestations occurred as the result of increase in numbers of an indigenous population breeding on originally clean goods, whereas the *Oryzaephilus* had been introduced on the dried fruit from abroad.

A survey of the main problems during and after the war period

(*a*) *Sources of infestation*

The origin of infestations is of considerable importance in the preparation of a sound plan of control and prevention. If wrong conclusions are reached much control work may be misdirected. The infestations which occur in commodities in this country arise in two principal ways. First, there is a constant flow of infested commodities from abroad; these goods have already become infested in the countries of origin, and in many cases the insects continue to develop in the goods during storage in this country. Other goods, perhaps free from infestation or only lightly infested, may become more heavily infested by contact in ships or other transport with infested goods. In addition, clean goods may be carried in ships which have previously carried infested commodities. Secondly, commodities which arrive clean in this country may become infested from the endemic populations in stores during the period of storage; they may become infested by contact with other infested commodities or by passage through infested machinery (e.g. in flour mills).

(*b*) *Infestation of imports*

Since 1942 a high proportion of all cargoes of susceptible commodities arriving at United Kingdom ports has been examined in the holds of the ships. Evidence has been obtained regarding the incidence of infestation of commodities from various parts of the world and, *inter alia*, a number of species of insects and mites new to science or not hitherto recorded in this country, have been intercepted. Some of these records have been published by Hinton (1944, 1947).*

* At this stage one may refer to a problem which arose early in the development of the inspectorial organization. Although it was possible to establish the organization with a cadre of trained entomologists a number of the inspectors had only general zoological or botanical training. It was necessary to train the inspectors to identify the more common pests and also to provide them with a service for the identification of strange species. Dr H. E. Hinton, who was seconded from the British Museum (Natural History) for this purpose, produced alone, and in co-operation with others, a series of useful systematic works, the need for which arose out of the day by day work in the field (Hinton, 1941, 1943, 1945; Hinton & Corbet, 1943; Corbet & Tams, 1943).

The main results of the inspections are expressed in Figs. 1 and 2. It will be observed, as one would expect, that commodities from the warmer parts of the world are the most heavily infested. Although goods from Canada and the United States of America are generally clear or only lightly infested, the number of species intercepted is high. This is a measure of the degree to which ships tend to harbour insects remaining from previous voyages with infested goods. For example, during 1947, at least two cargoes of Canadian wheat were infested by insects remaining in the ship after previous cargoes of American maize carried from U.S.A. ports to the Near East. The subject is developed in more detail elsewhere (Freeman, 1948).

(*c*) *Infestation during storage*

One of the interesting facts which emerged from the study of the frequency of occurrence of insects in commodities on import was that the most commonly introduced pests are not those, generally speaking, which cause the most trouble to goods in store in this country. Thus *Ptinus tectus* Boield. (Australian spider beetle) and *Ephestia kühniella* Zell. (Mediterranean flour moth) are very seldom found in imported produce; *E. elutella* is much less common in imported goods than *E. cautella* Walk. (fig moth).

Ptinus tectus is our most common warehouse pest. It attacks a wide variety of commodities, breeding exceptionally rapidly on such foods as fishmeal and dried yeast. Apart from the actual destruction and fouling of the food itself, a heavy infestation by Ptinids may result in damage to bags and to the woodwork of stores by tunnelling of the larvae. It is of interest to note that *P. tectus* is an invader from Australia, the first record in this country being in London in 1892 (Chitty, 1904). *Ephestia elutella*, whose depredations on wheat have already been mentioned, also attacks a wide range of goods including stored tobacco, cocoa beans, flour, compound feeding stuffs, soya beans and soya products, and oilseeds. Following the 1941 outbreaks, the biology of this species was carefully studied by Richards & Waloff (1946), whose results have been of great value in devising appropriate techniques of control. In particular the observation that most of the eggs are laid during the first four days of female adult life, made it essential that protective film insecticide spraying should be carried out well in advance of emergence. Mites,* especially *Tyroglyphus farinae* L. (flour mite) and *Carpoglyphus lactis* (dried fruit mite), are serious pests. Mites not only cause losses by direct attack but also by fouling and tainting, leading to loss of food out of all proportion to the amount actually consumed. Severe infestations by mites occur in wheat, flour, animal feeding stuffs and oilseeds, particularly under conditions of high relative humidity and lack of ventilation. The biology of mites in the field has been studied by Solomon (1946*a*, *b*), whose method for the determination of relative humidity in small spaces by the use of papers impregnated with cobalt thiocyanate has been used regularly in the field by the inspectors (1945). *Calandra granaria* L. (grain weevil) and *Oryzaephilus surinamensis* L., which are well established in this country as well as being regularly introduced, are responsible for outbreaks of infestation in grain. In farm granaries they occur in grain held back for use by the farmer (incidentally it may be noted that stored products insects do not attack grain held in the stack). Although the total number of such farm granary infestations that have come to our notice is small, there is the danger that, with the increase of combine harvesting and the tendency of farmers to store grain in bulk, serious infestations may occur through lack of experience of the necessary precautions. In this connexion the recent book by Oxley (1948) should be of considerable assistance.

Heating. One of the phenomena associated with infestation of grain is heating. Until the work of Oxley & Howe (1944), it was thought that heating of bulk grain was due almost exclusively to general or local excessive moisture content, and that the presence of insects in

* For mites, as for insects, it was found necessary to provide an identification service for the inspectors. In the course of this work Mrs A. M. Hughes, of the London School of Medicine for Women, has discovered a number of new species and has completed a monograph on the mites of stored products, which will be published in 1948.

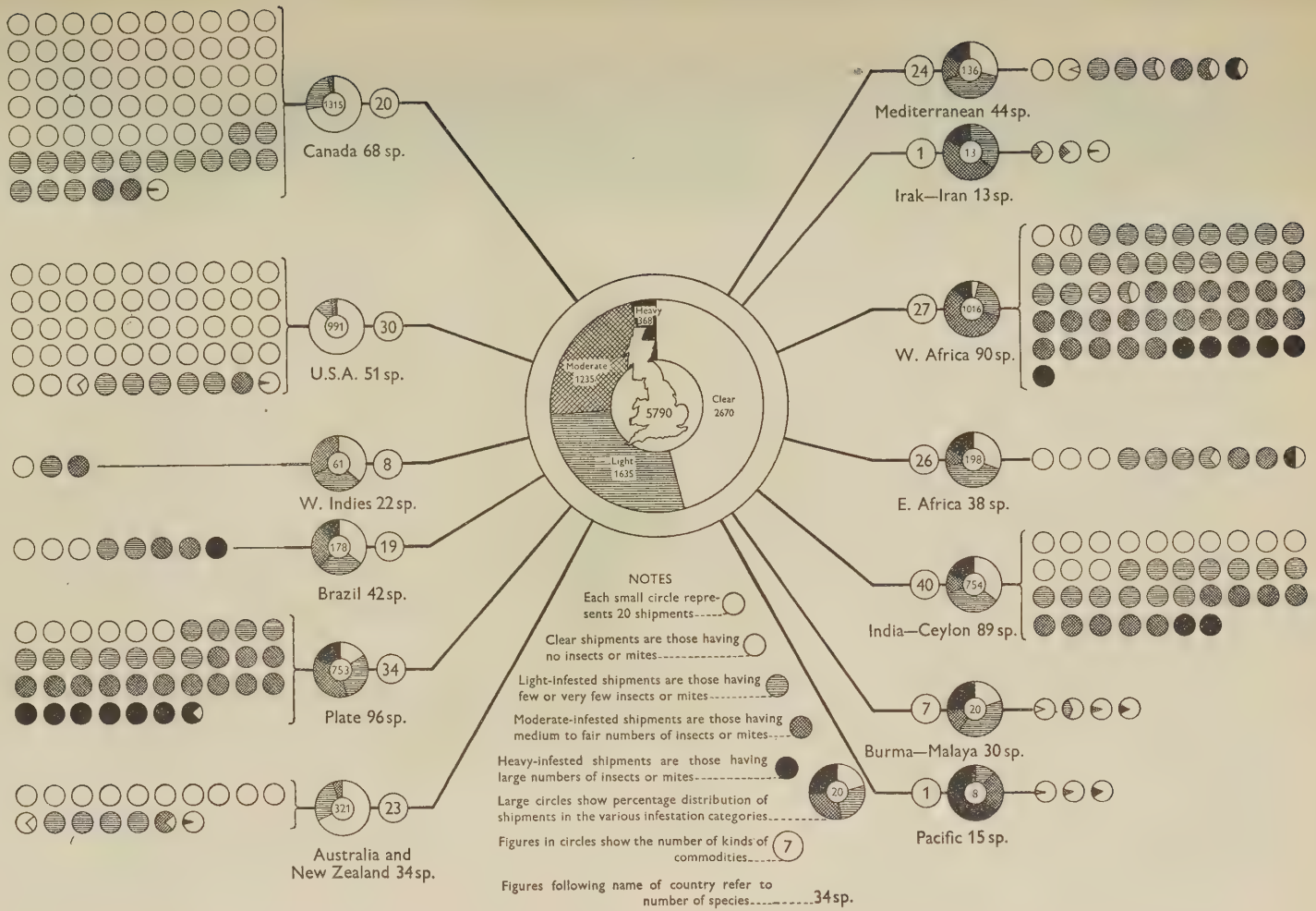

Fig. 1. Infestation of commodities imported into the United Kingdom during 1944, 1945 and 1946.

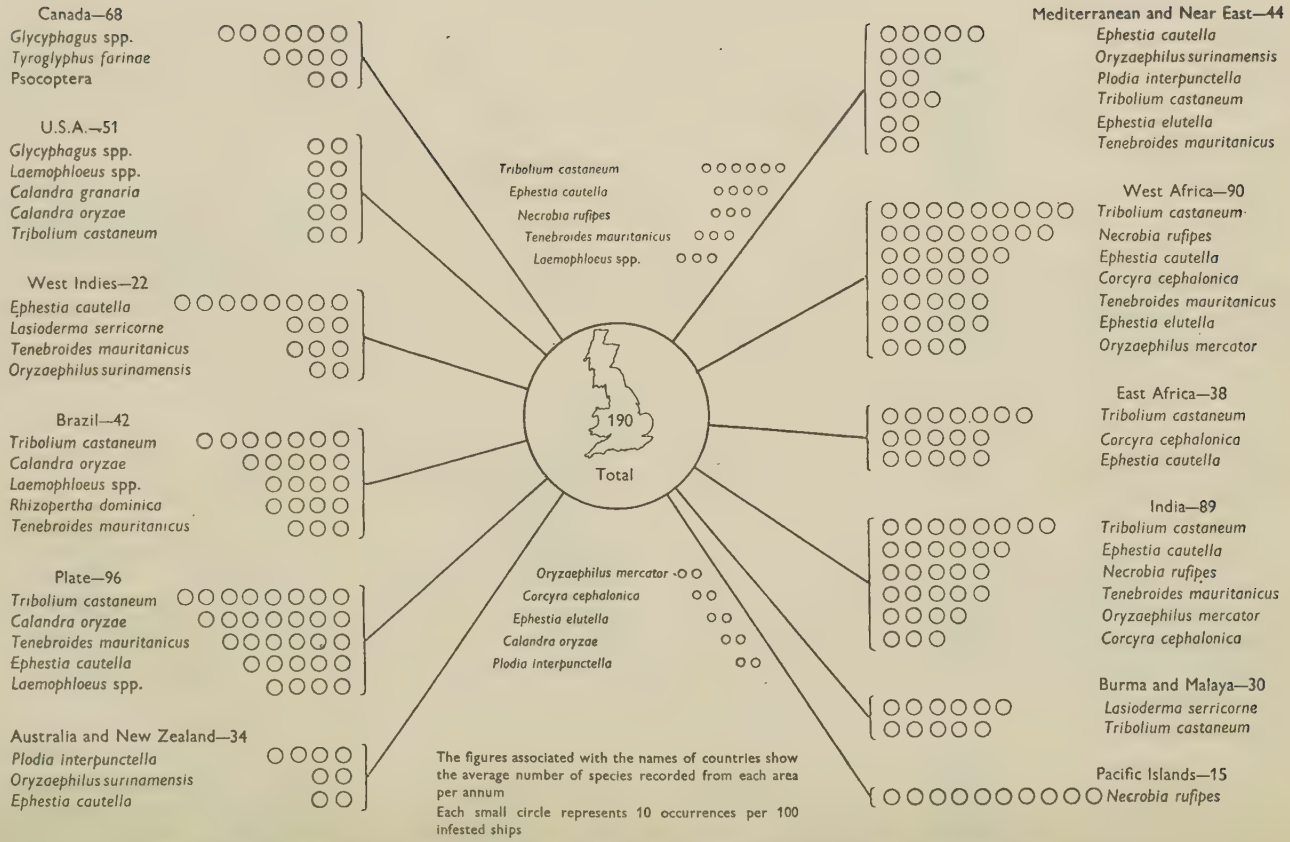

Fig. 2. The countries of origin of the insects and mites occurring most frequently on goods imported into the United Kingdom during 1944, 1945 and 1946.

large numbers in heating grain was the result of heating and not its cause. Their discovery of the mechanism by which quite small populations of insects can initiate heating was of great assistance in the discovery of infestation in the field and in the method of dealing with heating grain. So long as it was thought that excess moisture was the cause it was considered impossible to hold in store such heating grain without drying, although by turning or revolving grain stored in silos from bin to bin it was possible to keep the temperature down for a time. The principal objection to storage by the warehousekeeper was not so much the actual damage to grain by insects as the liability of the grain to cake and become immovable in the bin. Attempts were therefore made to fumigate heating infested grain, and success was obtained not only in killing the insects but also in converting a steady rise in temperature into a steady fall. Care had to be taken to check the moisture content, for, although the insects might be killed, the grain might have a sufficiently high moisture content to allow heating for other reasons. Sampling apparatus developed in the course of these studies has been adopted as part of the equipment of the inspectors (Oxley & Henderson, 1944) or used on occasion for investigation in special cases arising in the course of routine work (Lucas & Glover, 1946).

A further factor in this problem was the detection of infestations of sufficient intensity to cause such heating. Although the problem has not been fully solved, decisions to fumigate grain, particularly *ex* ship, have been made much easier by the adoption, as a routine measure, of the Oxley-Howe carbon dioxide production test, which is much more accurate and provides a considerable saving in time over dissection or breeding-out techniques (Howe & Oxley, 1944).

At first it was thought that such heating was caused only by grain-boring insects such as *Calandra* spp., *Sitotroga cerealella* Oliv., or *Rhizopertha dominica* L., but it was found, more or less simultaneously in this country and in Canada, that species of *Laemophloeus* could breed in the germ of wheat and cause sudden increase of temperature of a more serious character than that caused by other species (Lucas & Oxley, 1946).

(*d*) *Infestation during processing*

A number of beetles and moths are commonly found infesting the machinery of flour, provender, rice and other cereal mills. The major pest is *Ephestia kühniella*, whose larvae web together the stock passing through the machinery. If this webbed material is not cleaned out regularly it may cause blockages involving stoppage of the mill and loss of production. The larvae of *Ephestia*, of *Tenebroides mauritanicus* L. (cadelle beetle), of *Dermestes lardarius* L. (bacon beetle), may also eat holes in silk screens and tunnel into woodwork. This is all without taking into account the danger of infestation of finished products.

(*e*) *Infestation in warmed premises*

Many species of stored products pests are inactive or are killed by exposure to winter conditions in this country. In artificially heated premises, however, breeding may continue throughout the year. A serious pest in kitchens, restaurants, bakeries and hospitals is *Monomorium pharaonis* L. (Pharaoh's ant), which nests in cracks and crevices. Detailed studies of the biology of the ant have been made by Prof. A. D. Peacock, University College, Dundee; one of the results has been to show that a period of several months is necessary for the elimination of colonies by poison baiting.

Although some of the common warehouse pests, e.g. *Ptinus tectus* and mites, do not breed readily under warm conditions, others thrive. During the war, flour was stored in cotton mills, which were heated to protect the machinery from deterioration. There were several outbreaks of *Ephestia kühniella* and *Tribolium castaneum* L. (rust-red flour beetle) in parts of cargoes stored there, whereas other parcels from the same ships stored in unheated premises were free. In one former retail shop which was heated, flour became heavily infested by *Tineola biselliella* Hummel (webbing clothes moth), a cross-infestation from textiles. One of the most serious pests which thrive under hot dry conditions is *Trogoderma granarium* Everts

(khapra beetle) which attacks malt. The insect is difficult to eradicate because of the habit of the larvae of penetrating deep into crevices and of their ability to remain for long periods without food. The pest is so feared by maltsters that the finding of a few larvae in a consignment of malt may be sufficient to cause a maltster to lose a high proportion of custom.

Main lines on which research might profitably be directed

Whereas certain species, e.g. *Calandra granaria* and *Ephestia elutella*, have been studied in detail, there are many common pests whose life history has been little investigated. It should be possible, over a period of years, to arrange that each of the species found associated with stored products should be studied so that the essential basic information is obtained. The species studied should not be solely those already found in this country but should include potential pests not so far established, such as, for example, the spider beetles, *Ptinus villiger* Reitt and *Ptinus raptor* Sturm, which are serious pests in Canada under climatic conditions similar to those in this country. The work should cover the following main categories:

Systematics: Life history (under both laboratory and field conditions).

Ecology, with special reference to over-wintering, the effects of high and low temperatures and geographical distribution.

Behaviour, with special reference to the movements of insects in goods and buildings and their diurnal activity (Ewer, 1943).

Nutrition, both in regard to the types of commodity attacked and basic food requirements, as studied by Fraenkel & Blewett (1943).

Population problems, particularly interspecific competition and host-parasite and predator relationships, bearing in mind the possibility of using biological control under certain conditions.

A general subject of great importance is the *economic loss* due to pest attack. With stored products pests in particular, the direct losses due to the depredations of the insects or mites often represent only a minute fraction of the total economic loss. There are few reliable figures for losses and it would be well worth while to carry out a systematic investigation.

Conclusion

Although stored-products entomology was relatively neglected before the war there are signs that far more attention is being given to the subject at present. Not only has the Government established an organization for research into the subject in the Pest Infestation Laboratory of the Department of Scientific and Industrial Research, and an executive control organization in the Infestation Control Division in the Ministry of Agriculture and Fisheries and the Department of Agriculture for Scotland, but the Colonial Office is conducting surveys in Africa, and the Food and Agricultural Organization of the United Nations, largely under stimulus from the United Kingdom, has set up a small organization for the dissemination of information.

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STORED-PRODUCTS PESTS: A SURVEY OF METHODS OF CONTROL IN THE UNITED KINGDOM SINCE 1939

By E. E. Turtle, *Ministry of Agriculture Infestation Division, 58 High Holborn, London, W.C. 1*

Since the beginning of the 1939–45 war, changes in storage, transport and processing of foodstuffs have demanded new methods of pest control. The methods then available in this country were derived either from the Imperial College investigations, e.g. the use of pyrethrum sprays in warehouses or fumigation with ethylene oxide, or from work carried out mainly by commercial interests in U.S.A. or Germany. Such procedures were the use of liquid and of absorbed hydrogen cyanide which were employed here by commercial servicing companies who did not maintain research or development sections in Great Britain. It is now evident that the techniques available here, and their practical application, were considerably behind those in various foreign countries; we now know, for example, that there were many grain silos in Germany already equipped with gas circulation apparatus in 1939, whereas in this country there were none.

Treatment of grain

In view of the importance of grain reserves in war time, treatment of stored grain infestations soon became a first charge on workers in this country. Precise knowledge was not available on the causes of deterioration of stored grain and, to the practical silo man or grain warehouseman, the detection of heating was the main criterion of safe storage. He knew that wet grain would heat, and also that insects were in some way associated with the occurrence. Work on insect control was assigned to the newly-formed Pest Infestation Laboratory of the D.S.I.R. where the possibilities of using (*a*) dusts and (*b*) gases formed the main lines of approach.

Insecticidal dusts

As no insecticidal chemical of likely efficacy and without toxic properties to human beings was available in sufficient quantity, consideration was mainly directed to the inert dusts whose action was apparently based on physical rather than chemical properties. These investigations were stimulated by reported uses abroad, for example, in Germany and the Middle East, and by the availability of promising materials. Although the laboratory studies provided much information (Parkin, 1944; Fitzgerald, 1944; Alexander, Kitchener & Briscoe, 1944) the use of the dusts in this country never passed beyond the stage of pilot trials. It was found to be very difficult to achieve adequate admixture under commercial conditions without incurring undue risks from inhalation of the dusts; and the degree of control secured in previously infested grain was most disappointing. This was an important point, as our main problem was to treat infested grain coming into the country.

Gassing of grain

No silos equipped for gas circulation or even suitable for hermetical sealing, each of which was common in different parts of the Continent, existed in this country; nor did the situation allow for such construction. The possibilities of direct use of the gases commercially available, viz. ethylene oxide and hydrogen cyanide was therefore considered. Chemical measurements of their penetration soon confirmed that artificial means of distribution would be essential for the treatment of grain in bulk.

Use of ethylene oxide and carbon dioxide 'slush'

This mixture, of about 7 parts solid carbon dioxide with 1 part of liquid ethylene oxide, of consistency similar to melting snow had been described previously for treating grain in silo bins (Back, Cotton, Young & Cox, 1930). The mixture is added in small quantities to the grain entering the bins. However, no data were available on the behaviour of the gaseous components in grain or of their toxicity to the different species encountered. Queries whether the treatment would leave harmful residues or otherwise damage the grain also had to be cleared up in the laboratory before field trials could be conducted.

These trials included the measurement of concentrations of gas in the working spaces during operations and in the intergranular spaces in the grain. They revealed a number of important practical objections. The behaviour of the two gases was found to be different: the ethylene oxide was sorbed appreciably and did not readily diffuse from localities which had received the mixture to others which had not, whereas the cold carbon dioxide, which was hardly sorbed at all, passed down the bins, readily leaking away altogether. In operation the method was messy and sufficient concentrations of gas were displaced from bins during the treatment to require masks to be worn for periods of work. These objections may have been overcome had not alternate means of treatment become available.

Granular calcium cyanide

Although this method had previously been used abroad, important queries, relating both to its efficacy and the dangers of contaminating grain, had to be answered before it could be employed under practical conditions in this country. This preliminary work, which was undertaken principally at the Pest Infestation Laboratory but also in association with the Imperial College and the Flour Millers' Research Association, included investigations of sorption of HCN by grain, methods of chemical estimation of residues, assessment of baking damage, together with measurements of concentrations of gas obtained from different rates of admixture and the toxicity of these concentrations to different species of insect. Only when a considerable amount of laboratory work had been conducted were field trials undertaken. These were first directed at assessing the operational hazards; for example, by measuring the concentrations of HCN present in the air displaced from bins during the course of treatment, and measuring concentrations of gas evolved from grain on delivery from treated bins; but the efficacy of the process was also assessed by measuring concentrations of gas present inside bins after treatment.

Since this time the method has been developed into practice and employed, almost on a routine scale, in many silos throughout the country. Apart from occasional chemical checks, results are normally assessed by direct inspection and by observations of the arrest of heating after treatment. Judged on these standards, a high level of success has been achieved. However, interesting limitations of the method have been revealed by investigation of individual failures. At low intergranular humidities, i.e. grain below approximately 11% moisture, the reaction $CaCN_2 + H_2O \rightarrow Ca(OH)_2 + 2HCN$ is far from complete and calcium cyanide remains unreacted: at high humidities, i.e. greater than approximately 17%, the micro-organisms which cause heating are not appreciably affected by the relatively low concentrations of HCN present.

The method has been used on a large scale because it offers a means of tackling our main problem in this field, which is to treat the grain at the port silos. However, in addition to these restrictions, grain often has to be specially turned for treatment, which may be a slow and costly operation; furthermore, it must stand for a period of about 10 days, which may be serious in a congested port silo. To meet these difficulties the use of methyl bromide for the treatment of standing bins is under close investigation.

Owing to the fact that immediate control is required at a point in transit, i.e. at the port silo, the new solid insecticides in powder, granular or other form do not appear to offer the

potentialities of gassing methods. In fact we still have no hesitation in advocating the installation of gas circulatory systems into port silos.

Treatment of cased and bagged goods

The necessity of obtaining rapid control and of achieving dissemination of insecticide are also reasons likely to retain fumigation as the principal method of treating these commodities.

In this country before the war, fumigation of bagged, baled and cased goods was employed commercially to control individual outbreaks and on a small scale. This work was carried out on a purely empirical basis and without critical data. Owing to the detailed work of Page and Lubatti on dried fruit, however, the current practice with this commodity was based on a considerable background of exact data. This was particularly concerned with distribution of gases, and penetration into commodities, but it also included basic information on the nature of sorption, airing, size and importance of residues (Page, 1932; Page & Lubatti, 1933, 1937; Brown, 1937). This form of approach has been accepted as the model for tackling the many subsequent problems in this field. A body of data has thereby been built up on which it is possible to advise dosages and techniques of fumigation of a wide range of commodities to give effective results without risk of damage and without waste of insecticide.

Many types of commodity have been treated. They include grain, beans, oil seeds, meals and flours, empty sacks and bags, and also cased and baled goods such as dried fruits and hides. Treatments are mainly conducted in barges and in well-constructed warehouses: a little is in chambers although these are not common in this country. Occasionally treatments of stacks below gas-proof sheets are also carried out. Ethylene oxide with carbon dioxide, and hydrogen cyanide evolved from inert absorbent, have mainly been used for this work. Each of these possesses advantages for particular purposes although both are sorbed appreciably. In application they are also restricted by their poor penetration which, for example, prohibits the effective treatment of dense stacks of commodities. Methyl bromide substantially meets these objections and therefore has been recently brought into practical use (Brown & Lewis, 1946). It enables commodities to be treated successfully almost irrespective of density of stacking: for example, barges loaded with grain in bulk are acceptable. However its introduction into practice is limited by its high toxicity to human beings (Anon., 1946) and the precautionary measures therefore necessary when using it. Owing to this factor and its high rate of penetration, which includes leakage from structures, methyl bromide does not seem likely to displace HCN for the treatment of large empty premises such as flour mills. For the latter type of treatment in fact we do not consider any of the newly introduced gaseous, or other insecticides are likely to have substantial effect on the established practice of fumigating with hydrogen cyanide.

Spraying

The dangers of leaving toxic residues, tainting or otherwise damaging foodstuffs, are factors of outstanding importance in this field and greatly restrict the choice of spraying material: questions of fire hazards and dangers to operators, who may be employed for long periods, are also important. The material developed by Potter at Imperial College before the war, consisting of a solution of pyrethrins in medicinal paraffin (Potter, 1935), satisfies safety requirements and has been used extensively.

The spraying is normally applied with paint spraying equipment. The techniques of application are broadly (1) as a mist, (2) as a direct spray, and (3) as a lasting film. The choice between these depends first on the biology of the insect but also very largely upon other factors such as commercial practices with different commodities (e.g. whether they are stored for long periods), condition of premises, nature of surfaces. These conditions vary with cases and, more often than not, cannot be altered appreciably by the applied entomologist.

The first method is not used extensively, being restricted because the insecticide is rapidly ventilated away from most premises: many of the species do not fly and some that do start to

oviposit a few hours after emergence. To control such species therefore, mists, which only affect the adults, must be present almost continuously during the emergence period. The direct spray technique is employed where the insects are present on the surface, e.g. on walls of a warehouse after delivery of infested goods, on to migrating larvae of *Ephestia elutella*. Used in this way, pyrethrum spraying has not failed against any of the species of insect encountered. The object of the third technique, which has been used extensively, is to leave a film on all available surfaces with which insects are likely to make contact (Potter, 1938). Although this has given many good results it is far from universally applicable: apart from specific aspects such as the marked lack of effect of pyrethrum films against certain species, e.g. spider beetles, there are objections due to the difficulties in obtaining effective films on many of the surfaces encountered in practice, e.g. walls covered with whitewash or plaster. Various methods, including pre-treating with sealing materials (Hewlett & Parkin, 1945), use of emulsions or of dispersible powders, have been suggested or are under investigation for overcoming the latter difficulties. Other restrictions to the present technique are the relatively high costs of material and labour.

Use of D.D.T. and B.H.C.

It seems appropriate to consider these in dealing with problems arising from the use of the film technique. Largely because of the doubts regarding the possibilities of leaving harmful residues under practical conditions, the amount of exact knowledge of their efficacy in stored product work is small. It is therefore only possible to indicate certain properties likely to restrict or to favour their application.

Their properties do not seem to offer any marked advantages over pyrethrum for use in sprays. As mists, against flying insects, they cannot be used alone because their action is too slow. As direct sprays they do not produce the immediate arrest secured by pyrethrum. We have very little reliable information on the effects of residual deposits against stored-products pests, although we know that applications of solution on to absorptive surfaces are as ineffective as are those of pyrethrum. Their relative inefficacy against lepidopterous larvae is another important restrictive factor in a field in which these insects are important.

Their main potentiality appears to be as dusts for protection of stocks of grain, e.g. as barriers, for treating empty premises and for dusting surfaces; this applies particularly to B.H.C. because of its high specificity to *Calandra* spp. Difficulties in obtaining satisfactory mixing of dusts with grain have been mentioned already, as have the advantages of fumigation for obtaining an immediate arrest of insect activity, which is required by circumstances in this country. Apart from such technical aspects, however, overriding questions of risk to human beings are likely to restrict direct admixture to the treatment of seeds and other grains not for human consumption. Such restrictions are likely to remain until the authorities can feel satisfied that adequate safeguards are available against the risk of leaving toxic quantities in foodstuffs. In the case of B.H.C., tainting is an additional restrictive factor, although, owing to the very small amounts of the γ-isomer necessary for effective results, this is not always prohibitive. This applies particularly if the purified γ-isomer is used. Other important uses not possible with the unstable insecticides formerly available are the impregnation of bags and structures and the inclusion of insecticides in devices such as smoke generators, which overcome many difficulties in application.

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REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1947

The Officers and Council of the Association were as follows:

President: W. C. Moore, M.A.

Vice-Presidents: C. B. Williams, M.A., Sc.D.; R. W. Marsh, M.A.

Hon. Treasurer: H. F. Barnes, M.A., Ph.D.

Hon. Secretary: H. Shaw, M.Sc., Ph.D. (until April); G. V. B. Herford, O.B.E., B.A., M.Sc.

Hon. Asst. Secretary: J. R. Busvine, B.Sc., Ph.D.

Hon. Editor of The Annals of Applied Biology: R. W. Marsh, M.A.

Hon. Asst. Editor: I. Thomas, M.Sc., Ph.D.

Council: M. Cohen, Ph.D.; H. J. Craufurd-Benson, Ph.D.; J. A. Freeman, Ph.D.; Miss M. D. Glynne, D.Sc.; Prof. F. G. Gregory, D.Sc., F.R.S.; D. Price-Jones, Ph.D.; W. G. Keyworth, Ph.D.; J. T. Martin, D.Sc.; R. T. Pearl, B.Sc.; D. Snow, Ph.D.; F. G. D. Thomas, B.Sc.; R. L. Wain, Ph.D.

The Council is able to report another year of good progress for the Association. The membership has continued to increase, 61 new members having been elected during the year. The total membership, including 11 Honorary Members, now stands at 486, a new high figure, and an increase of 56 over the previous year's total.

It is with regret that the Council have to record the deaths of Miss Enid Moore and Prof. R. S. MacDougall, an original member. Miss Moore was mycologist at the Tobacco Research Laboratory, Balfour, Cape Province. (An obituary notice of Prof. MacDougall appears in this number.)

Six well-attended meetings were held in London during 1947, an average of 45 members and 31 visitors recording their names as being present on each occasion.

The meeting on 25 April took the form of a Conversazione, held in the Botany Department, Imperial College of Science. A large number of exhibits were on view, and there was also a display of scientific films. There was a very large attendance, and it is hoped that similar meetings may be held from time to time in the future.

The Summer meeting, held on 20 June, consisted of a day spent in the Evesham district, with visits to the Plant Pathology Laboratory, Evesham, and the Fruit and Vegetable Preservation Research Station, Chipping Campden, by kind invitation of the Directors of the two institutions.

The following papers were presented to the Association during the year:

21 February. Presidential Address: The field of research in preventive entomology. Dr C. B. Williams.

21 March. Symposium on biological problems of the leather industry. Sir Robert Pickard: Introduction. Miss M. Dempsey and Miss M. E. Garrod: Some problems associated with collagen, reticulin and elastin in the skin. K. G. A. Pankhurst: Some aspects of the surface chemistry of animal skin. Miss M. Dempsey: The microscope and leather manufacture. J. N. Turner: Influences modifying the growth and quality of animal skin. A. J. Musgrave: Mycological and entomological problems of the leather industry. Miss M. E. Robertson and Miss B. M. Haines: Bacteria in leather manufacture. E. W. Merry: Enzymes in the leather industry.

3 October. Symposium on problems associated with the potato. J. G. HAWKES: Some observations on South American potatoes. W. BLACK: Blight in relation to potato breeding. A. R. WILSON: Potato storage in North America. C. E. FOISTER: A survey of Scottish seed tuber problems. A. E. W. BOYD: Some recent results of potato dry rot research.

7 November. A. C. EVANS: The biology of earthworms and their effects on the soil.

5 December. E. S. BUNTING: Oil seed crops. K. HOLLY: Control of weeds in oil seed crops. S. G. JARY and K. CARPENTER: Insect pests of Brassica seed crops. J. R. BUSVINE: The mosquito eradication campaign in Sardinia (illustrated by a film).

The Council wishes to take this opportunity of expressing its thanks to the authorities of the Imperial College of Science and Technology for so generously affording the Association accommodation for its meetings.

The Science Library's plan for the photocopying of articles in scientific journals was considered by the Council, who agreed to waive part of their copyright for an experimental term of two years. It was decided that from 1 January 1948 to 31 December 1949 the Science Library should have the consent of the Association of Applied Biologists to reproduce photographically and to sell without restriction the copies thus reproduced of any paper appearing in a volume of the *Annals of Applied Biology* of which publication was completed not less than five years previous to the date of photocopying. At the end of the trial period of two years the operation and effects of the scheme are to be reviewed.

PLANT PESTS AND DISEASES COMMITTEE REPORT FOR 1947

Four meetings have been held during the session, on 22 March, 24 April and 4 July 1947 and 19 February 1948. Miss M. A. Keay, S. H. Bennett, P. H. Gregory and I. W. Selman were appointed by the Council to fill the vacancies caused by the retirement under the rules of Miss A. P. Wilson, S. D. Garrett, H. C. Gough and H. G. H. Kearns (after two years), and A. C. Evans was appointed to fill the vacancy caused by the resignation of G. L. Hey (after one year). At the first meeting, J. T. Martin was re-elected Chairman and A. C. Evans was elected Secretary.

The Sub-Committee formed in collaboration with the Ministry of Agriculture Conference of Advisory Entomologists has made further progress in the compilation of the list of common and scientific names of British insect and other pests. Of Part 2 of the list, the orders Lepidoptera and Hemiptera have been completed.

The Committee has considered and reported upon the question of the recruitment and training in Great Britain of plant helminthologists. As a result, the Ministry of Agriculture proposes to hold a training and revision course for helminthologists during 1948.

The Committee has continued to assist the Plant Pathology Committee of the British Mycological Society in the definition of terms used in plant pathology, and has dealt with matters arising in connexion with virus nomenclature, the nomenclature of forest trees and the conservation of the specific epithets of fungi of economic importance.

HON. EDITOR'S REPORT FOR 1947

To meet the general desire for a return to a more readable format, Council authorized the adoption of the single-column page and 11-point type for vol. 34 of *The Annals of Applied Biology*. The volume consisted of pp. xi + 651 and 16 Plates, as against pp. viii + 465 (double-column) and 13 Plates for vol. 33. Including papers published in *Proceedings*, vol. 34 contained 58 communications, 46 being by members of the Association. The subjects may roughly be classified as follows: general applied botany, 11; mycology and fungus diseases, 7; viruses and virus diseases, 9; general zoology, 8; entomology and insect pests, 9; applied microbiology, 3; insecticides and fungicides, 11.

The demand for the *Annals* continued to increase and necessitated a further rise in the printing order to 1200 copies.

Publication delays were approximately the same as in 1946, and the several numbers of vol. 34 were issued on the following dates: no. 1, 7 June 1947; no. 2, 1 September 1947; no. 3, 25 November 1947; no. 4, 12 February 1948. In collaboration with the Cambridge University Press, a statement was drawn up and submitted to the Royal Society in reply to their questionnaire on the present state of the publication of original scientific papers in the United Kingdom.

The Association is participating in the Biological Council's scheme for distributing separates of Contents Lists of certain biological journals. A meeting of editors of these journals has made progress towards implementing another proposal of the Biological Council, namely, that a selected list of recommended abbreviations of titles of commonly used biological journals should be compiled and published.

The Editor expresses his thanks to the Assistant Editor and members of the Publications Committee who have responded so willingly to the considerable demands that have been made on them in 1947. The Editors and the Publication Committee take this opportunity of registering their appreciation of the efforts that certain authors have made to adapt their papers to present-day exigencies. They must, however, record that many contributors continue to disregard the Notice appearing on the back cover of each number of the *Annals*. A strict observance of the terms of this Notice would obviate much unnecessary labour, both in editing and in writing.

REPORT OF THE HONORARY TREASURER FOR THE YEAR ENDED 31 DECEMBER 1947

During the year ended 31 December 1947, the amount received for subscriptions and entrance fees, including arrears, was £574. 9*s*. 6*d*., which was £41. 5*s*. 0*d*. more than in 1946. The continuation of the steady rise visible during the last nine years is most satisfactory. Fifteen members were in arrears in the payment of their subscriptions at the end of the year.

Income from the sale of the current volume of the *Annals of Applied Biology* amounted to £1273. 8*s*. 6*d*., an increase of £196. 7*s*. 0*d*. on the year. During the year it again became necessary to raise the printing order from 1000 to 1200 copies. The volume contained 651 pages compared with 465 pages in 1946, and the cost of production rose by £216 to £2186. 7*s*. 6*d*. (The actual cost of printing the *Annals* was £2. 18*s*. 0*d*. per page compared with £3. 12*s*. 0*d*. in 1946, but this is a slightly exaggerated figure as it includes the cost (£122) of producing photographically 200 copies of Part 1 and the cost (£33) of reprinting 200 copies of Part 2 to bring the edition up to 1200 copies.) The income from the sale of back volumes

and parts was £238. 14*s*. 5*d*., a decrease of £108. 7*s*. 0*d*. compared with 1946. The sale of reprints showed a decrease of £27. The balance against the *Annals* on the year's working rose from £455. 8*s*. 11*d*. to £583. 3*s*. 7*d*., an increase of £127.

Income for the year showed a deficit of £133. 11*s*. 6*d*. over the expenditure. This is accounted for by the increased balance against the *Annals*, the net cost of producing the *List of Common Names*, Part 1, and the cost of the Conversazione as shown by the increases in the cost of printing and out-of-pocket expenses of the Officers involved, offset by the increase in subscriptions and interest.

The Association received from the Royal Society a grant of £140 in aid of publication and the sum of £1 from the Huddersfield Corporation towards the cost of printing a certain paper. Both these gifts are gratefully acknowledged.

After all obligations had been met, the net assets of the Association at the end of the year were £6. 8*s*. 6*d*. more than a year ago and amounted to £1738. 1*s*. 10*d*. Of this £801. 3*s*. 7*d*. was represented by National Savings Certificates and £126. 18*s*. 0*d*. by the estimated value of the stock of the *Annals*.

H. F. Barnes, *Hon. Treasurer*

REVIEWS

Five Hundred Varieties of Herbage and Forage Plants. Edited by M. HALL. Pp. viii + 316. Bulletin 39 of the Commonwealth Bureau of Pastures and Field Crops, Aberystwyth, Great Britain, 1948. 15*s*.

As mentioned in the introduction, this book attempts to assemble, for the first time, much relevant data concerning a wide range of varieties of plants used to provide animal fodder. It is inevitable that its format should be in the nature of a catalogue, and the result is a somewhat amorphous aggregate of detailed information. Even so, such aggregated detail rendered down to as uniform a pattern as the circumstances of a disturbed world permit, gives us, in a precise and ordered form, significant and welcome knowledge regarding the origin, adaptation, characteristics, and primary use of a multiplicity of crop plants from many countries. Of particular usefulness to isolated workers is the further information supplied, of availability of supplies, the source of each strain, whether stocks are certified or not, and the authority responsible for certification and grading.

Indeed, much of the merit of the volume is derived from the fact of data being fragmentary and incomplete, and from the knowledge that the treatment lacks complete uniformity. Such inadequacies stress the need for concerted world action on the naming of varieties and strains, reduction in the use of synonyms and the universal acceptance of standard schedules for crop plant descriptions on an agreed 'identity card' basis. Those responsible for the present publication need not be apologetic for the fact that information was not available for the main part of the work from large regions of the world. Such omissions include the U.S.S.R., and, as far as direct contributions for this issue are concerned, the U.S.A. also, as well as Denmark. It is hoped that such gaps will be filled later.

Rather do we feel thankful for finding in the main text of the work (pp. 1–295) authentic information direct from official correspondents and specialists in Australia, Canada, Finland, Great Britain, India, the Netherlands, New Zealand, Norway, Palestine, Sweden, South and East Africa and Trinidad. This information is arranged alphabetically according to genus and species, within species alphabetically according to country, within countries, alphabetically according to varietal name.

No less a boon to research workers, botanists and field husbandry men alike is the index (pp. 297–316). This contains exhaustive references to the volume and page in *Herbage Abstracts*, Vols. 1 to 17 (1931–47) on which information on a particular variety can be found. The references to Vols. 1 to 7 include a further refinement, since letters *a*, *b*, *c*, etc. indicate the siting of the relevant information lineally on any page. From Vol. 8 onwards, references are to volume and abstract number.

The reviewer has already found the work particularly useful in dealing with fodder cereals, in that the standard works usually listed in this field of crop plants often refer to grain-yielding varieties only.

When a reissue of the work becomes necessary, a few minor errors in established nomenclature and in spelling will need to be corrected. The work clearly fills a serious gap, of which we were all conscious, in collecting within two covers scattered information not otherwise readily available to workers outside liberally equipped large institutions; and it is hoped it will stimulate all workers to help to promote uniformity in standards, nomenclature and the use of synonyms in this field of applied studies.

It is highly desirable that this should be done, from scratch as it were, where, as in recent years, many crop plants hitherto put to other uses, are being tested for fodder and herbage use for the first time in order to meet the new outlook in an expanding live-stock economy the world over.

R. ALUN ROBERTS

A Textbook of Systematic Botany. By DEANE B. SWINGLE, Ph.D. 3rd ed. Pp. xv+343, with a frontispiece and 105 text-figures. New York and London: McGraw-Hill Book Company, Inc. 1946. 17*s.* 6*d.*

In this new edition of a well-known and most useful textbook most chapters have been thoroughly revised and amplified, and a new chapter on 'Methods used in the Identification of Flowering Plants' has been added. An elementary explanation of the newer species concept and the experimental method in taxonomy has also been included.

The chief merit of this book lies in its excellent treatment of the underlying principles of taxonomy and nomenclature; the author's statement in his preface to the first edition published in 1928, that 'no textbook is available that adequately sets forth the principles of taxonomy and nomenclature' is equally true to-day.

There are many textbooks in which a descriptive account of the Families of Flowering Plants is more fully and adequately treated than in this volume, but few in which more than a brief reference is made to the principles of taxonomy and nomenclature.

In the first edition, ten chapters on Principles and Methods constitute Part I of the book, and the Families of Spermatophyta are dealt with in the four chapters which form Part II.

In this edition there has been a change in the sequence of chapters as the actual study of plant groups is introduced earlier and is followed by chapters on Nomenclature, and Principles and Systems of Taxonomy.

This change will not, I think, be considered an improvement by many teachers of the subject, but it is not a serious defect as the chapters can be taken in almost any order after the first two have been studied.

This volume is not merely a textbook, but is of great value for advanced students, since it includes reports of recent taxonomic researches and a comprehensive list of references to taxonomic literature.

A. G. ERITH

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1947 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.
To Estimated Value of Stock, 1 January 1947	96	9	0	By Sales—Current Volume	1273	8	6
To Cambridge University Press	2186	7	6	By Sales—Back Volumes, Parts and Sets	238	14	5
				By Sales of Reprints and Adverts	60	12	0
				By Estimated Value of Stock, 31 December 1947	126	18	0
				By Balance, carried down	583	3	7
	£2282	16	6		£2282	16	6

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1947 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.	£	s.	d.
To *Annals of Applied Biology*, balance brought down	583	3	7	By Members' Subscriptions:						
To Printing and Stationery	29	0	5	Arrears	7	10	0			
To Postages and Cheque Stamps	14	11	5	Entrance Fees	25	14	6			
To Subscription—Parliamentary Science Committee	7	7	0	Current	541	5	0			
To Donation—The Biological Council	5	0	0					574	9	6
To Sundry Out-of-Pocket Expenses of Editors, Secretaries and Treasurer	45	15	8	By Sundry Income				1	0	2
To Expenses of Plant Pests and Diseases Committee	3	16	11	By Net Receipts—*List of Common Names*, Pt. 1				9	15	3
To Printing—*List of Common Names*, Pt. 1	79	10	2	By Interest Receivable:						
To Audit Fee	5	5	0	National Savings Certificates	25	8	9			
To Balance, being Excess of Income over Expenditure for the Year	—	—	—	Post Office Savings Bank Account	29	5	0	54	13	9
				By Balance, being Excess of Expenditure over Income for the year				133	11	6
	£773	10	2					£773	10	2

BALANCE SHEET, 31 DECEMBER 1947

Liabilities and Surplus	£	s.	d.	£	s.	d.	Assets	£	s.	d.	£	s.	d.
Sundry Creditors:							Cash at Bank:						
Cambridge University Press	653	12	7				Current Account	338	18	8			
Audit Fee Reserve	5	5	0				Post Office Savings Bank Account	1199	7	9			
Sundry Expenses	15	3	7								1538	6	5
				674	1	2	500 National Savings Certificates				801	3	7
Subscriptions and Entrance Fees, paid in advance				29	5	0	Stock of *Annals of Applied Biology* at estimated value				126	18	0
Life Membership Fund Reserve				25	0	0							
Excess of Assets over Liabilities:													
As per Balance Sheet of 31 December 1946	1731	13	4										
Add: Royal Society Grant	140	0	0										
	1871	13	4										
Deduct: Balance of Income and Expenditure Account for 1947	133	11	6										
				1738	1	10							
				£2466	8	0					£2466	8	0

H. F. BARNES, *Hon. Treasurer*

I certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to me, and, in my opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association according to such books, vouchers and documents.

(Signed) J. B. BENNETT, *Chartered Accountant* — *Auditor*

HARPENDEN, 17 *March* 1948